



#4

# Cleavage sites of endoproteases on the a-chain of GPIb

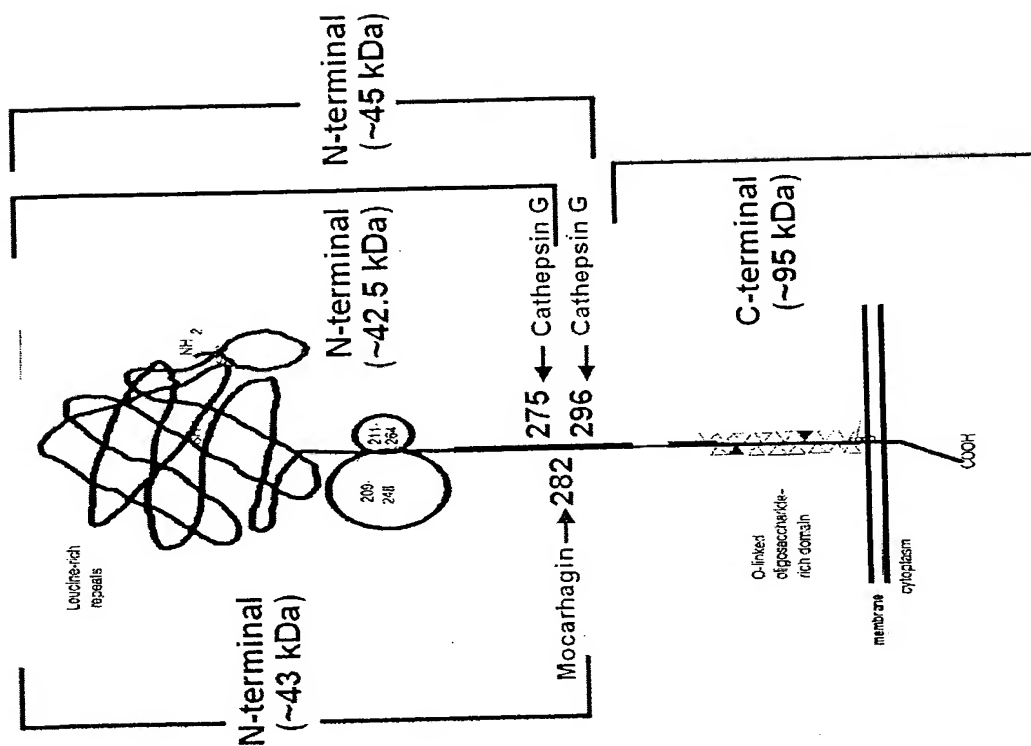
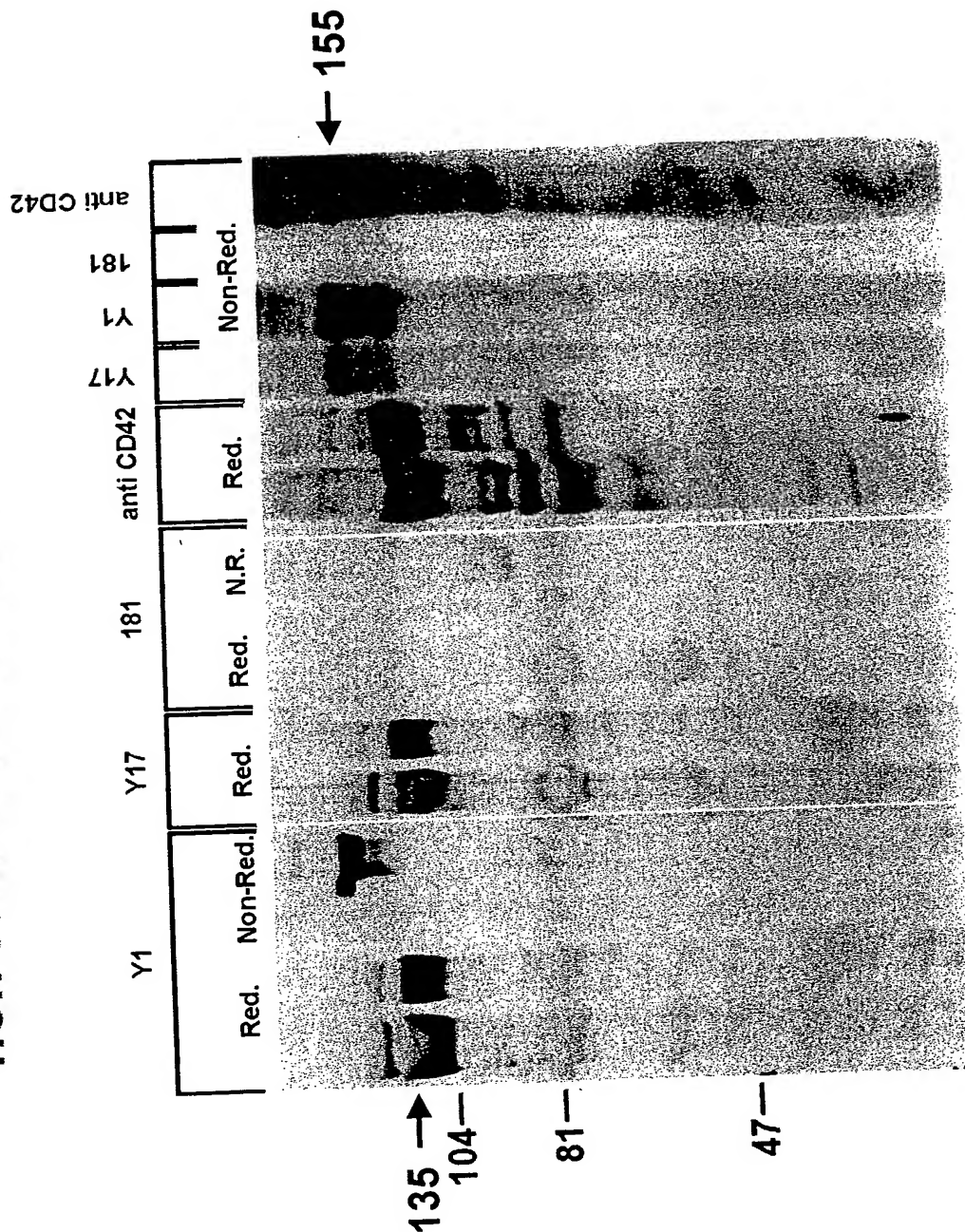


FIG. 1

FIG. 2

# Binding of Y1 and Y17 to platelets in reduced and non-reduced conditions



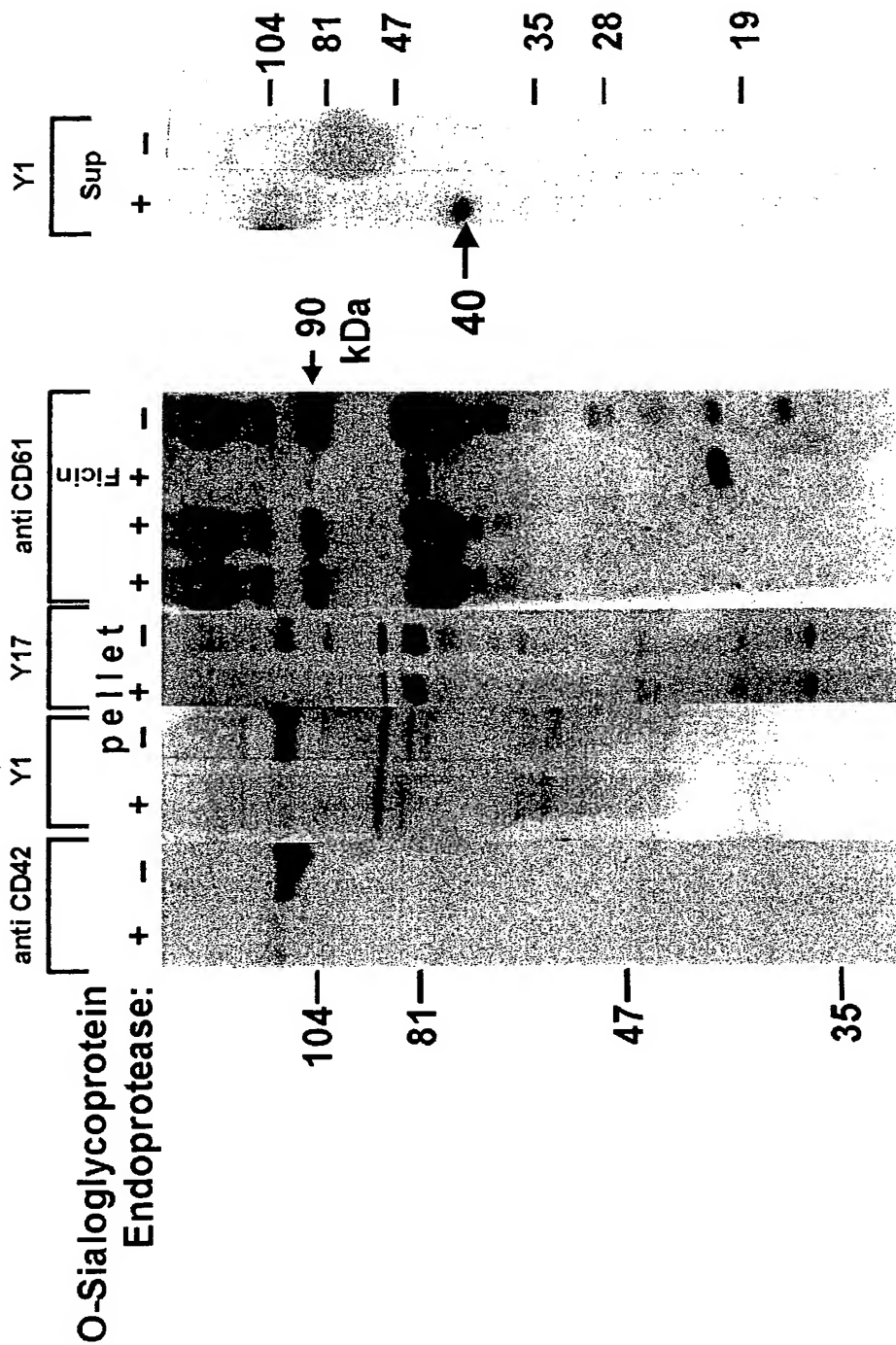


## Characterization of Optimal Determinants for Binding of Y1 to It's Ligands

**FIG. 3**

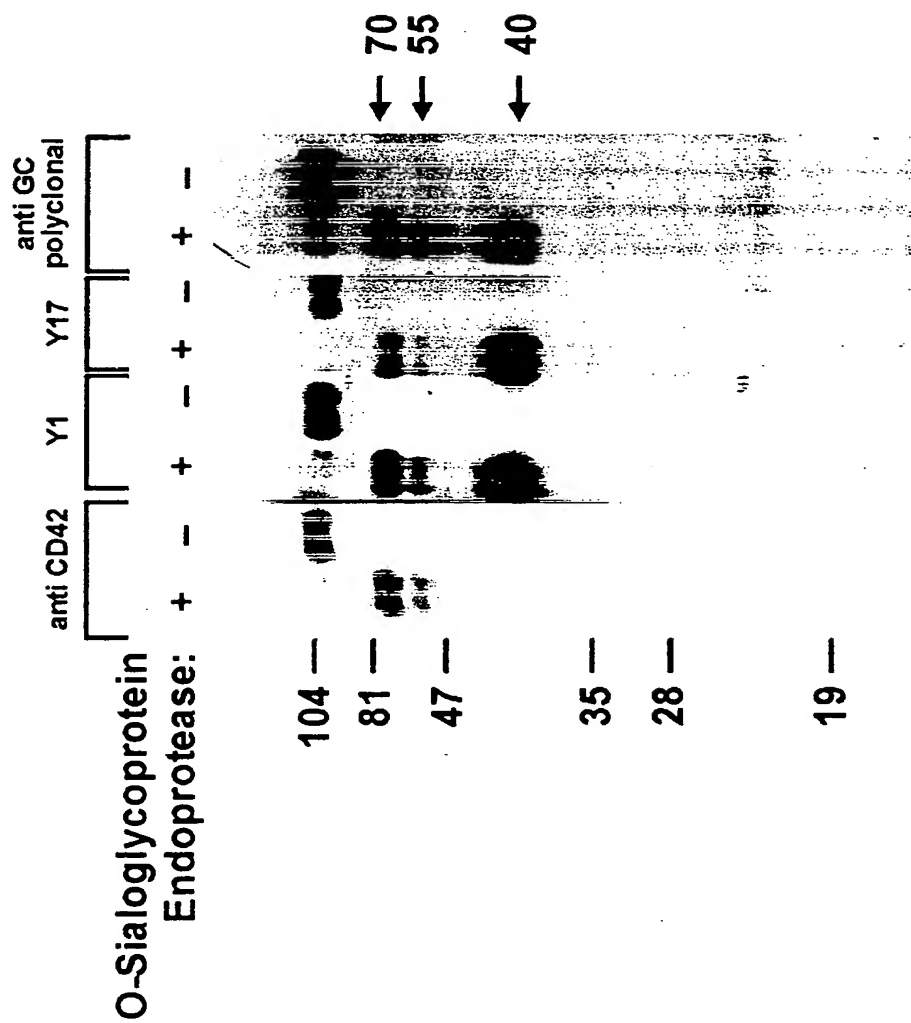
	Platelets/GC	KG1/RP-HPLC #4
Rec: GP1b 1-340 GP1b 1-480	- -	
Glycanase: N N+O	+ +	+++ +++
Proteases: Mocarhagin O-Sialo Peptidase Ficin Trypsine Elastase	++ (~40kD) ++ (~40kD) - ++ (~40kD) ++ (~40kD)	- - - - ++
Sulfatase (Aryl)		-/+

# Cleavage of platelets GPIb by O-Sialoglycoprotein abolishes binding of both Y1 and Y17



**FIG. 4**

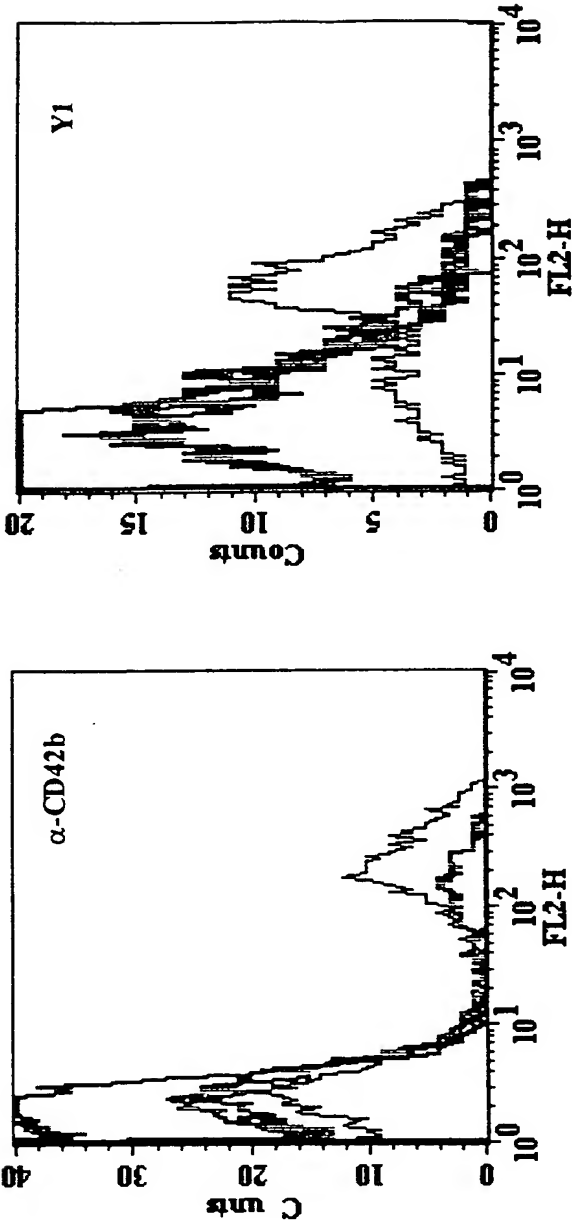
Y1 and Y17 binds similar glycosylated fragments after cleavage by O-Sialoglycoprotein Endoprotease



**FIG. 5**

Specific GPIIb Proteolysis Abolishes Y1 Binding to  
Platelets

FIG. 6

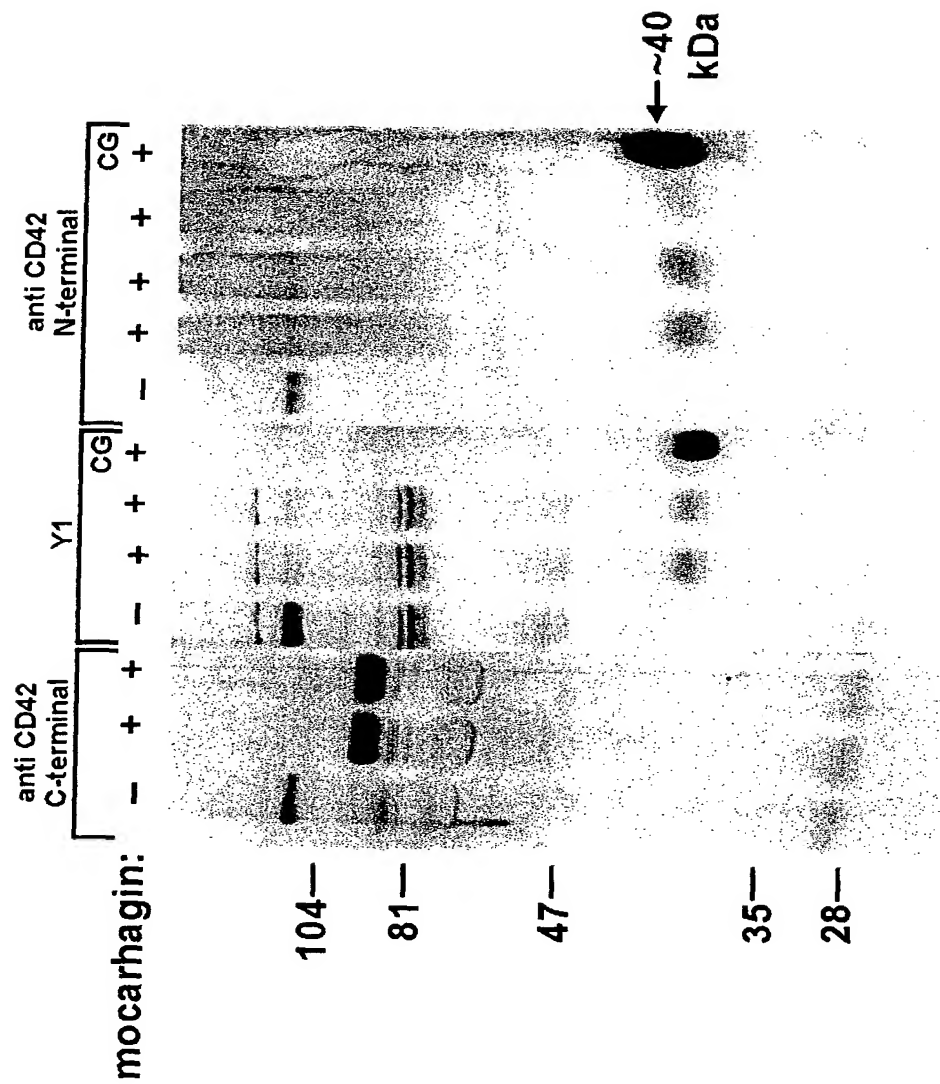


Key Name Parameter G

NON-TREATED PLATELETS

- O-SIALOGLYCOPROTEIN ENDO. (10 µg/ml)
- O-SIALOGLYCOPROTEIN ENDO. (50 µg/ml)
- FICIN (18 µg/ml)

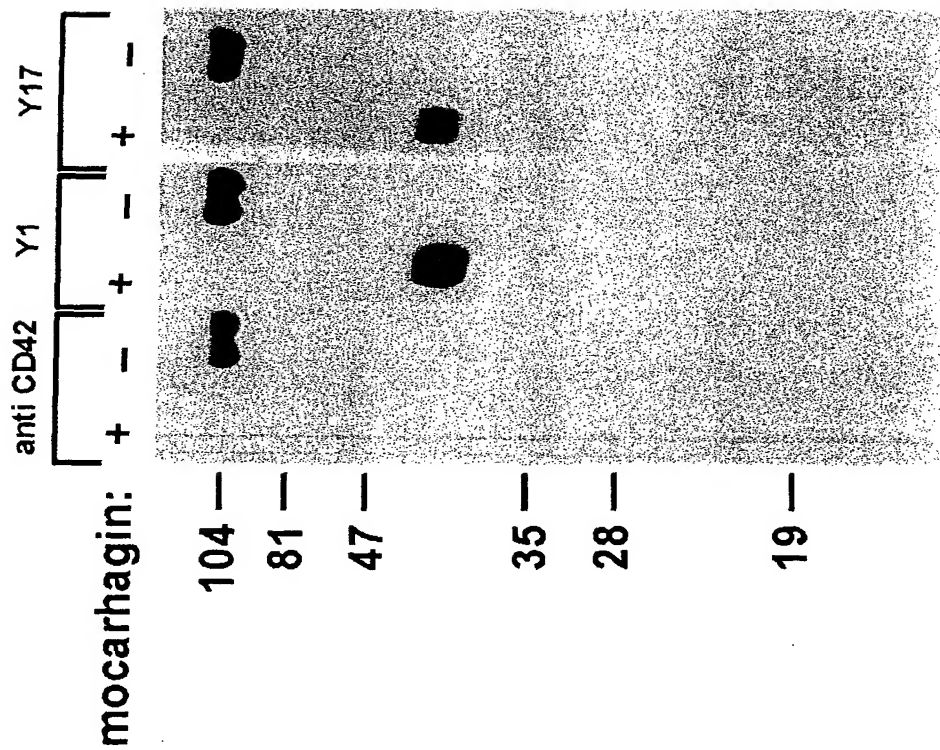
Y1 binds N-terminal (His-1 - Glu 282) fragment of platelet GPIb after cleavage by mocarhagin





**FIG. 8**

**Binding of Y1 and Y17 to glycolalycin after  
cleavage by mocarhagin**

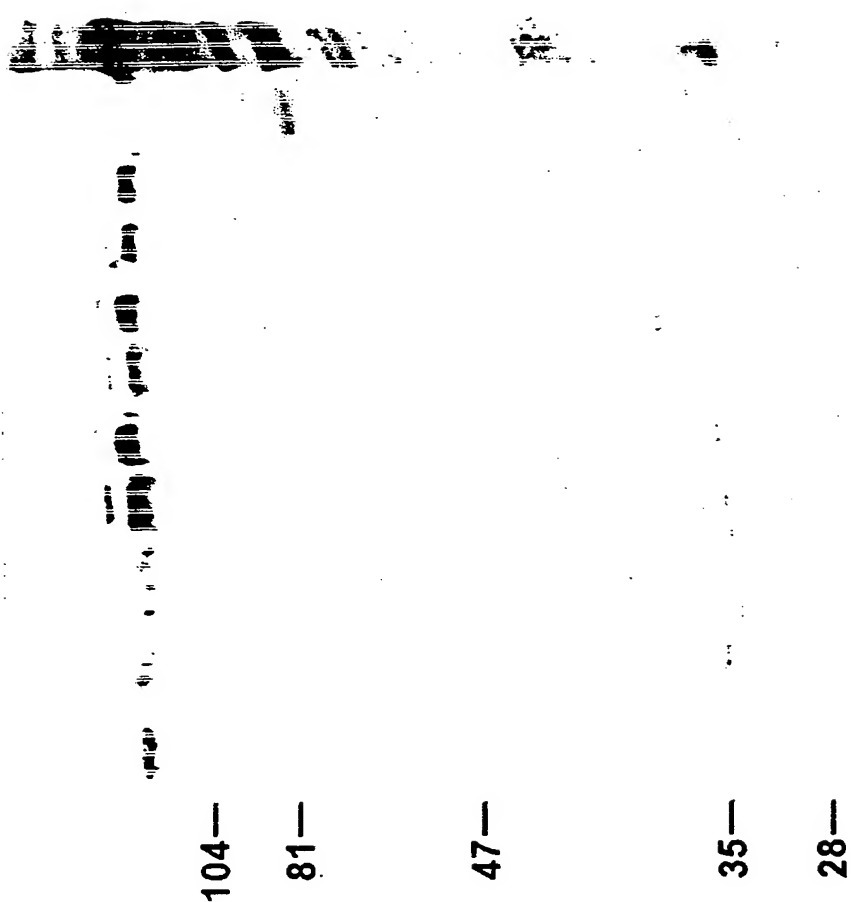
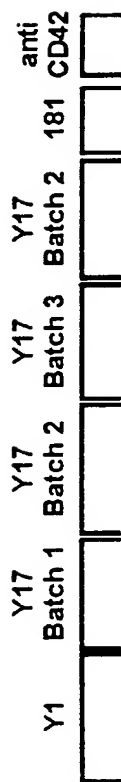


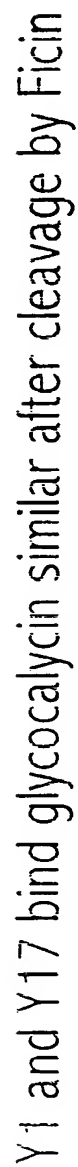




**FIG. 9**

**Binding of Y1 and Y17 to platelets**

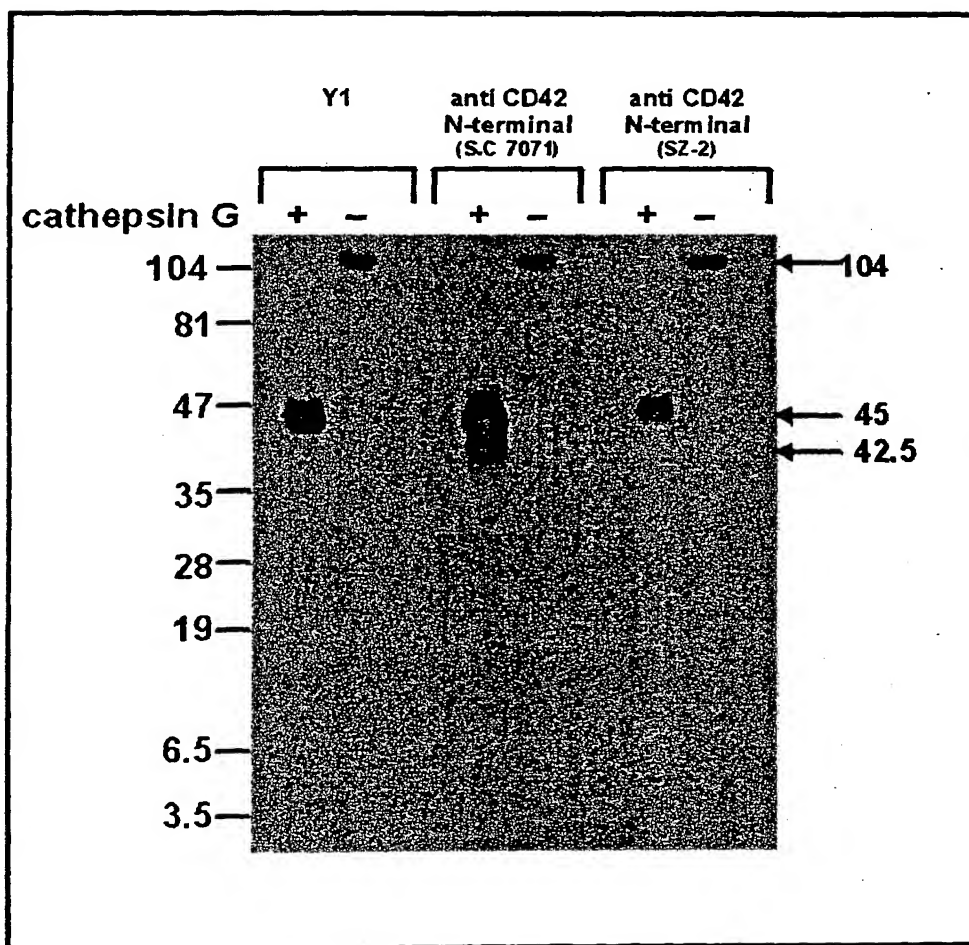




Ficin:	Y1		Y17		anti GC polyclonal
	-	+	-	+	
104	+	+	+	+	+
81	+	+	+	+	+
47	+	+	+	+	+
35	+	+	+	+	+
28	+	+	+	+	+
19	+	+	+	+	+

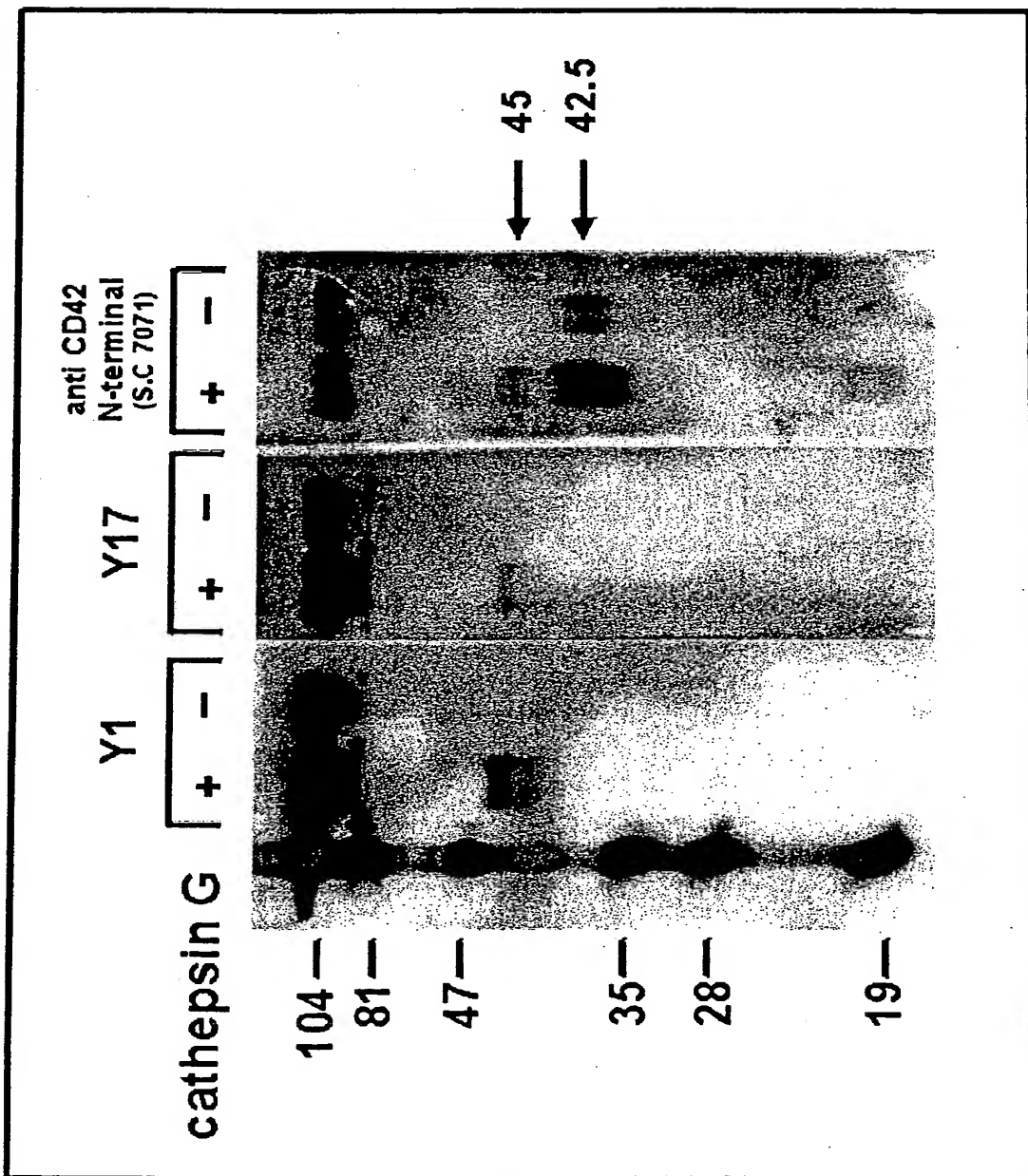


*FIG. 11*



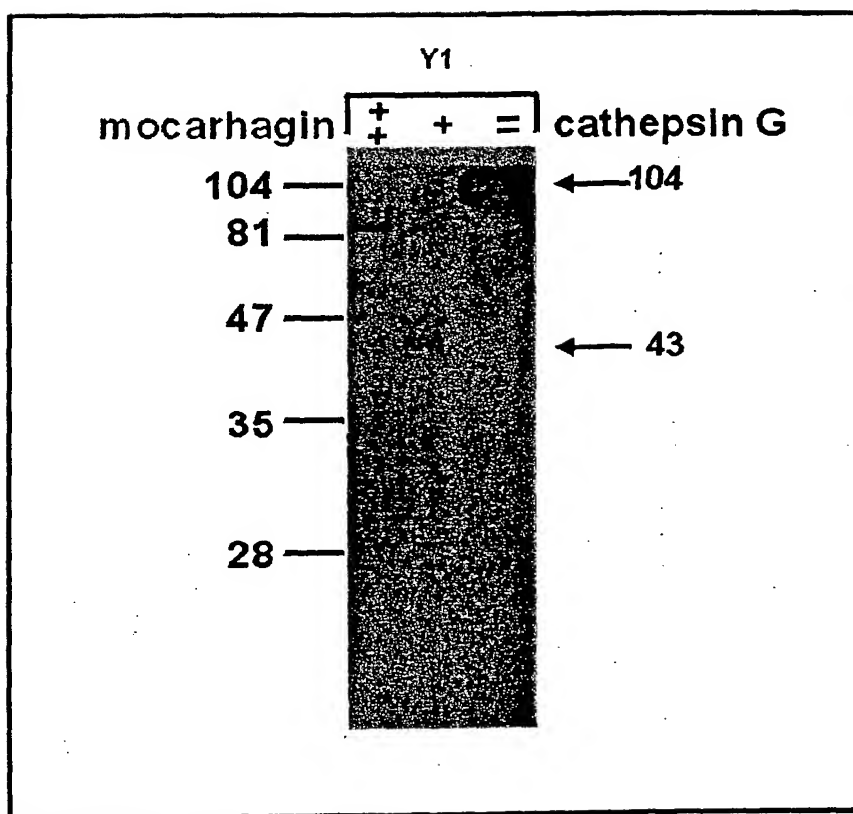
# Y1 and Y17 reacts with larger cathepsin G cleaved platelets GPIb fragment

FIG. 12





**FIG. 13**



# Cleavage of washed platelets by mocarhagin and cathepsin G

FIG. 14

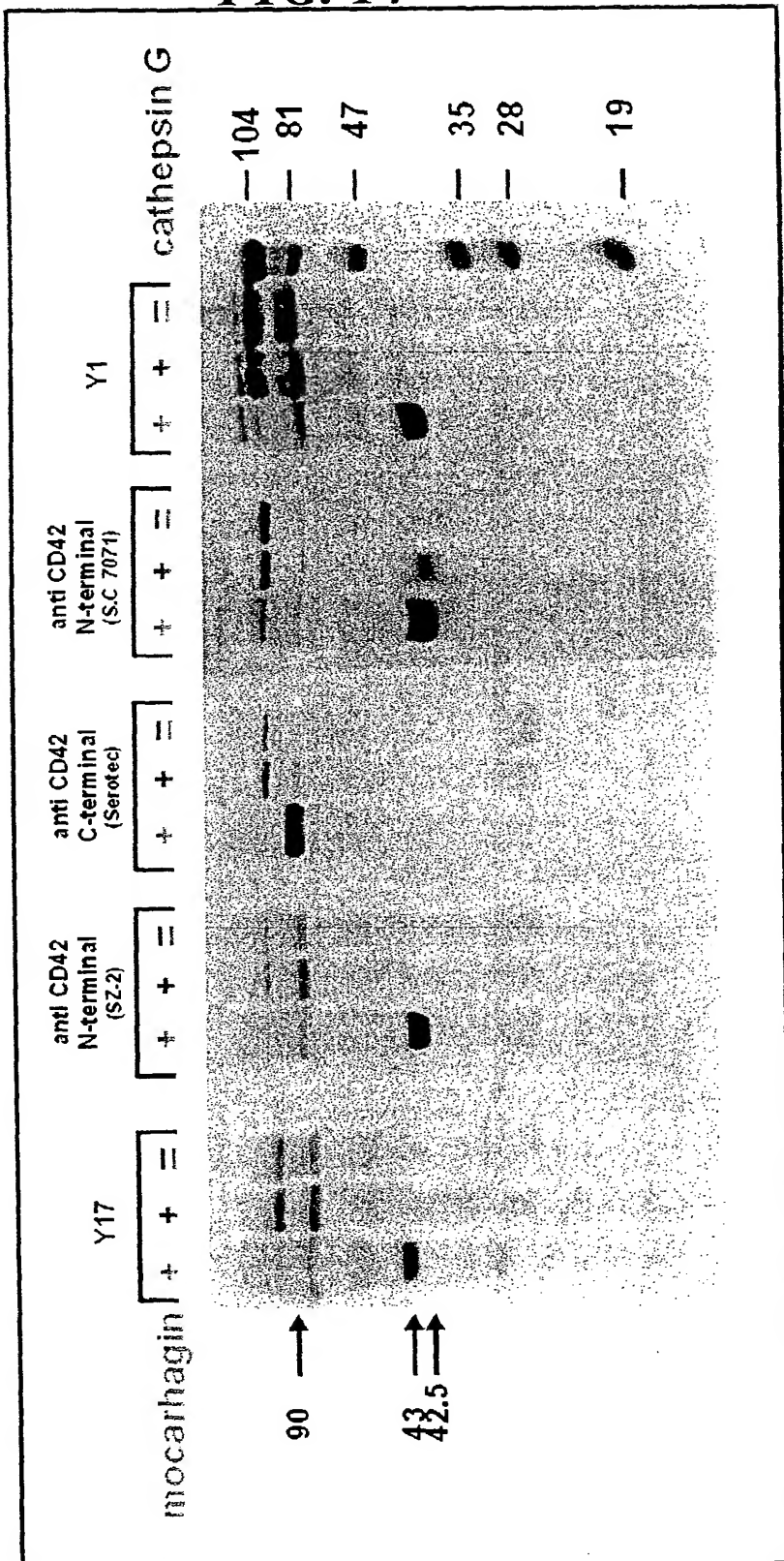
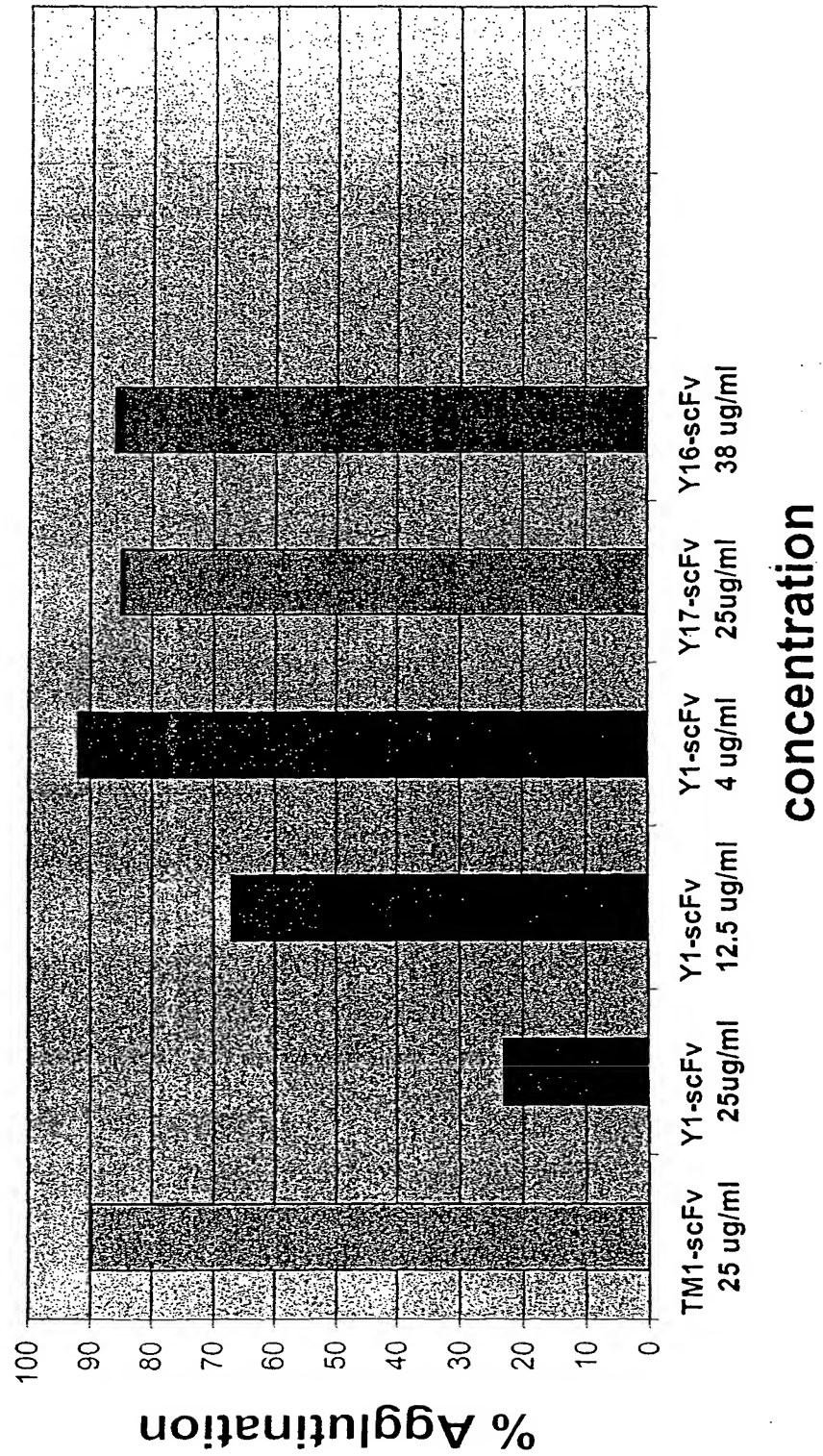


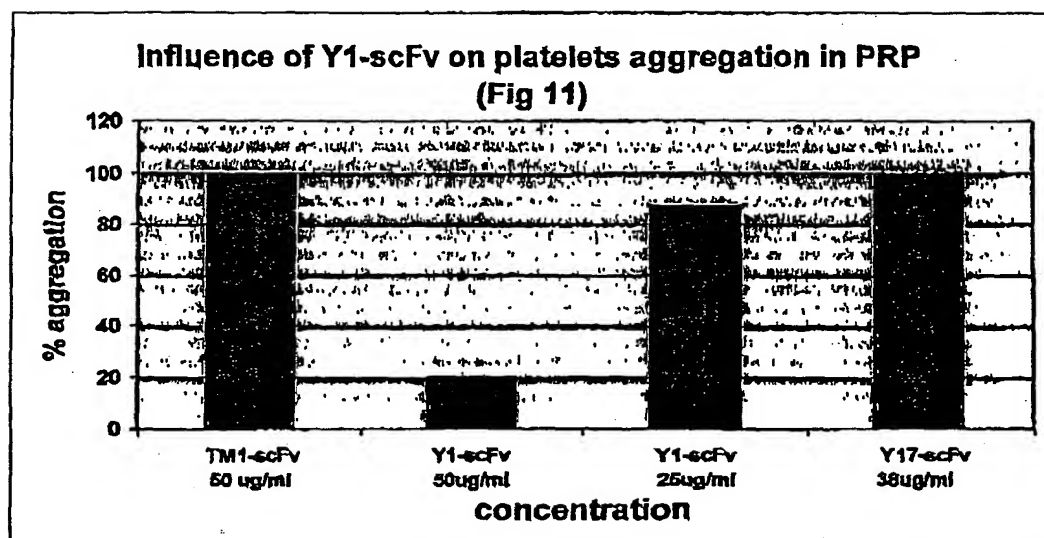
FIG. 15

Influence of Y1-scFv on platelets agglutination in washed platelets





**FIG. 16**





**FIG. 17**

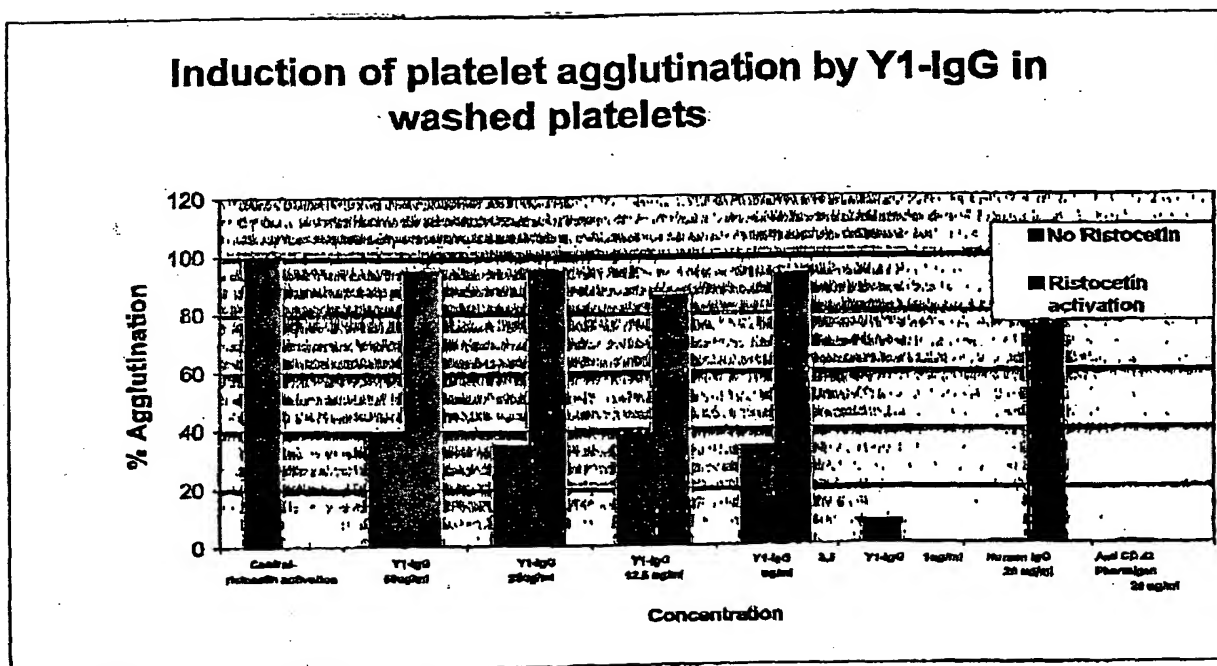
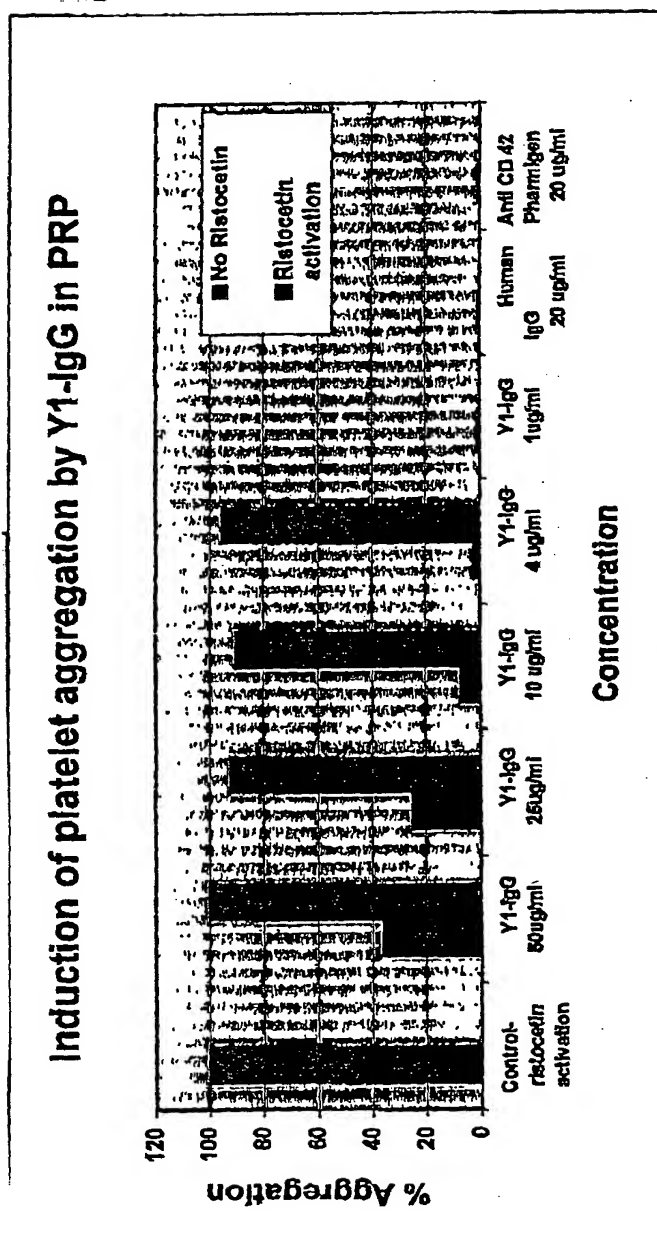


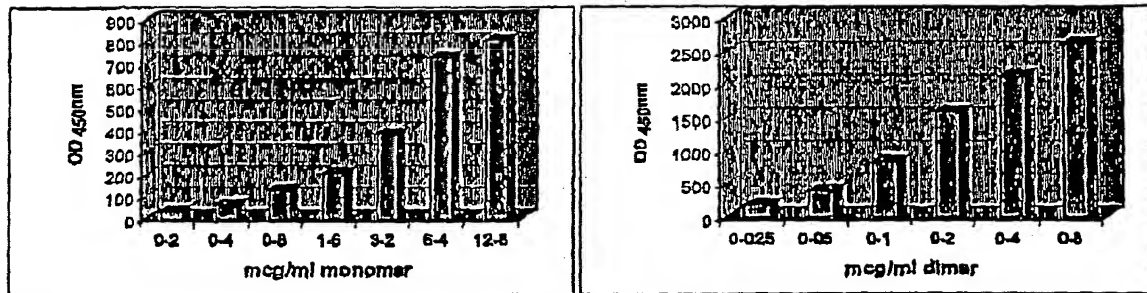


FIG. 18



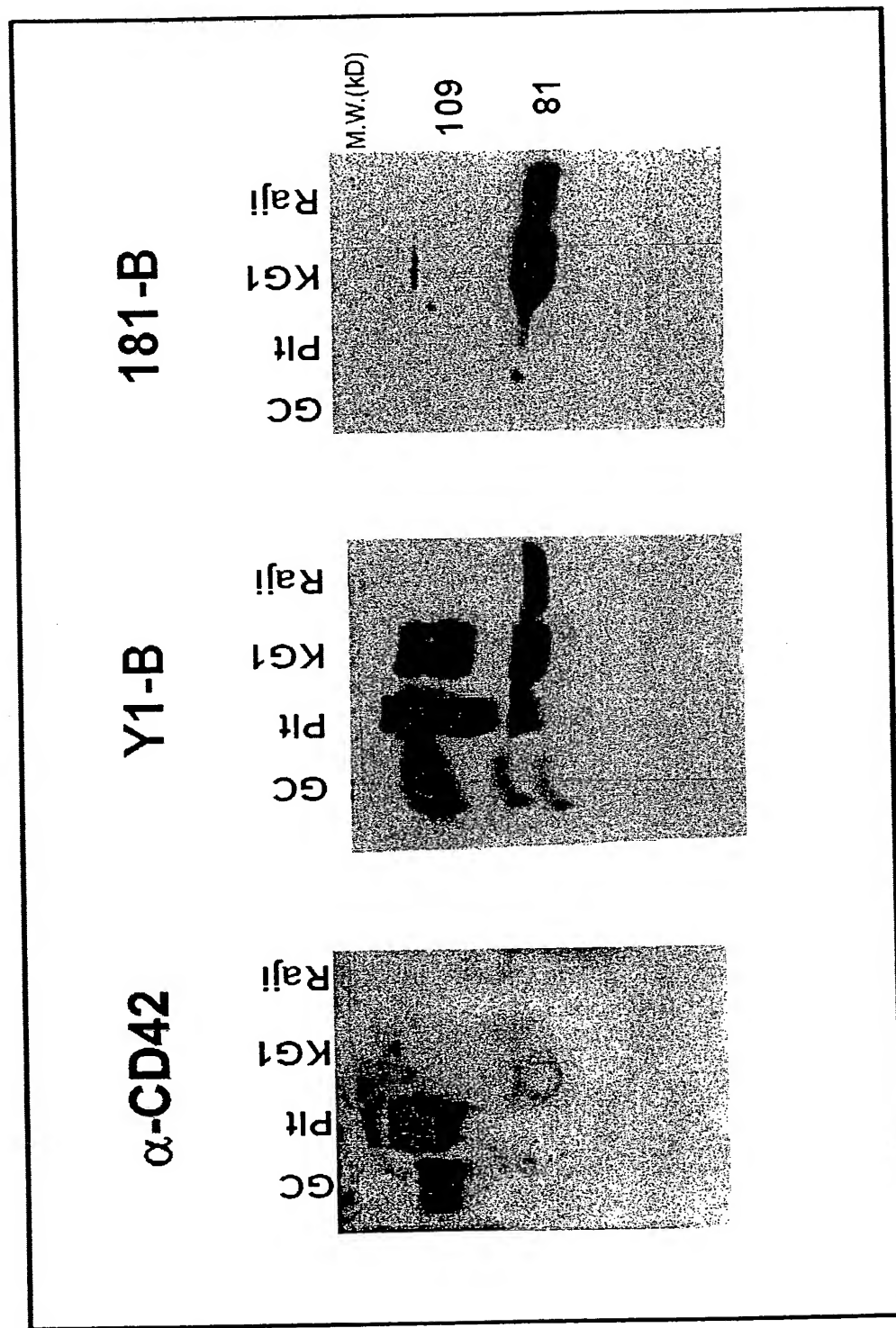


**FIG. 19**



# Specificity of Binding of Y1 and $\alpha$ -CD42 (N1-19) to their Ligands

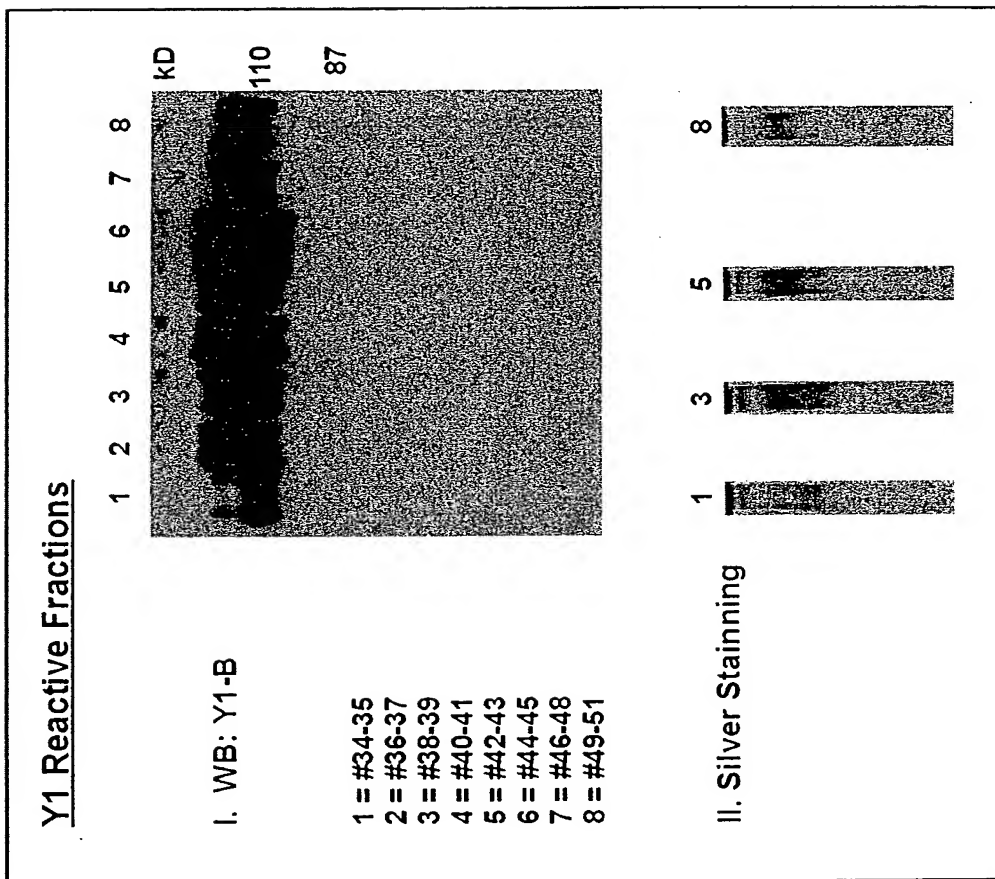
**FIG. 20**





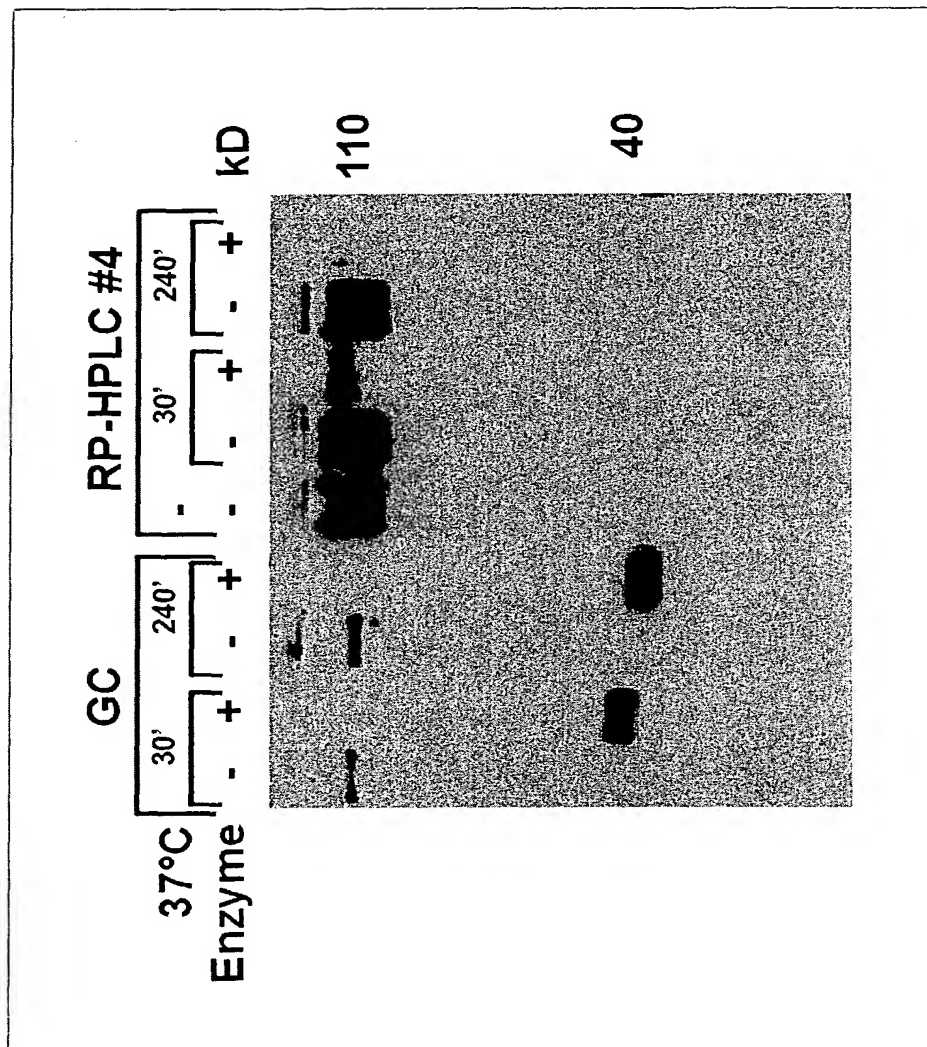
Y1-Ligand from KG1 membranes following  
Immuno-Precipitation with Y1:  
Purification on RP-HPLC

FIG. 21



# Effect of O-Sialo-Glycoprotein Endopeptidase on Y1 Binding

**FIG. 22**



# Effect of Aryl-Sulfatase on Binding of Y1: RP-HPLC(KG1) & H-B(Heparin-BSA)

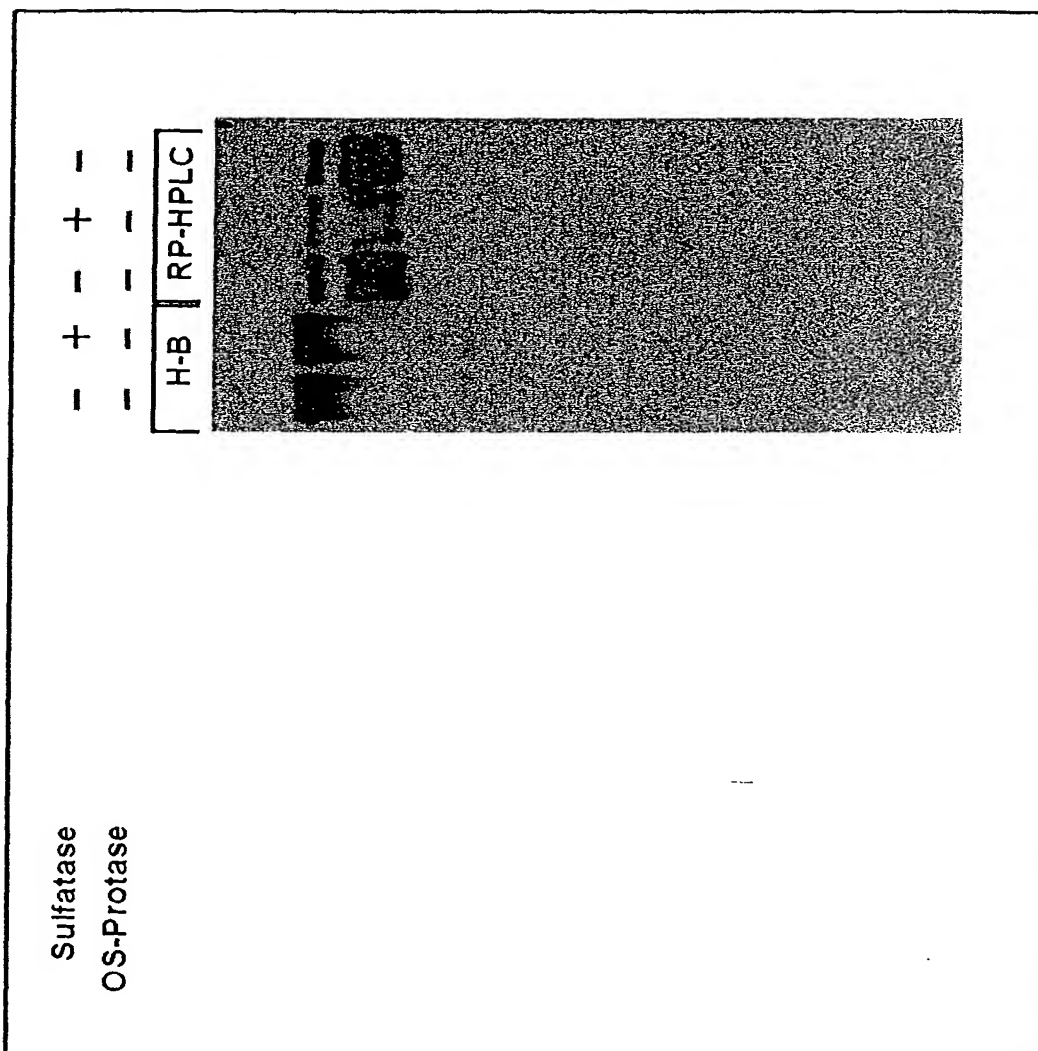


FIG. 23

# Specificity of Y1 Binding: Analysis by Immune Precipitation with Y1 and anti-PSGL-1

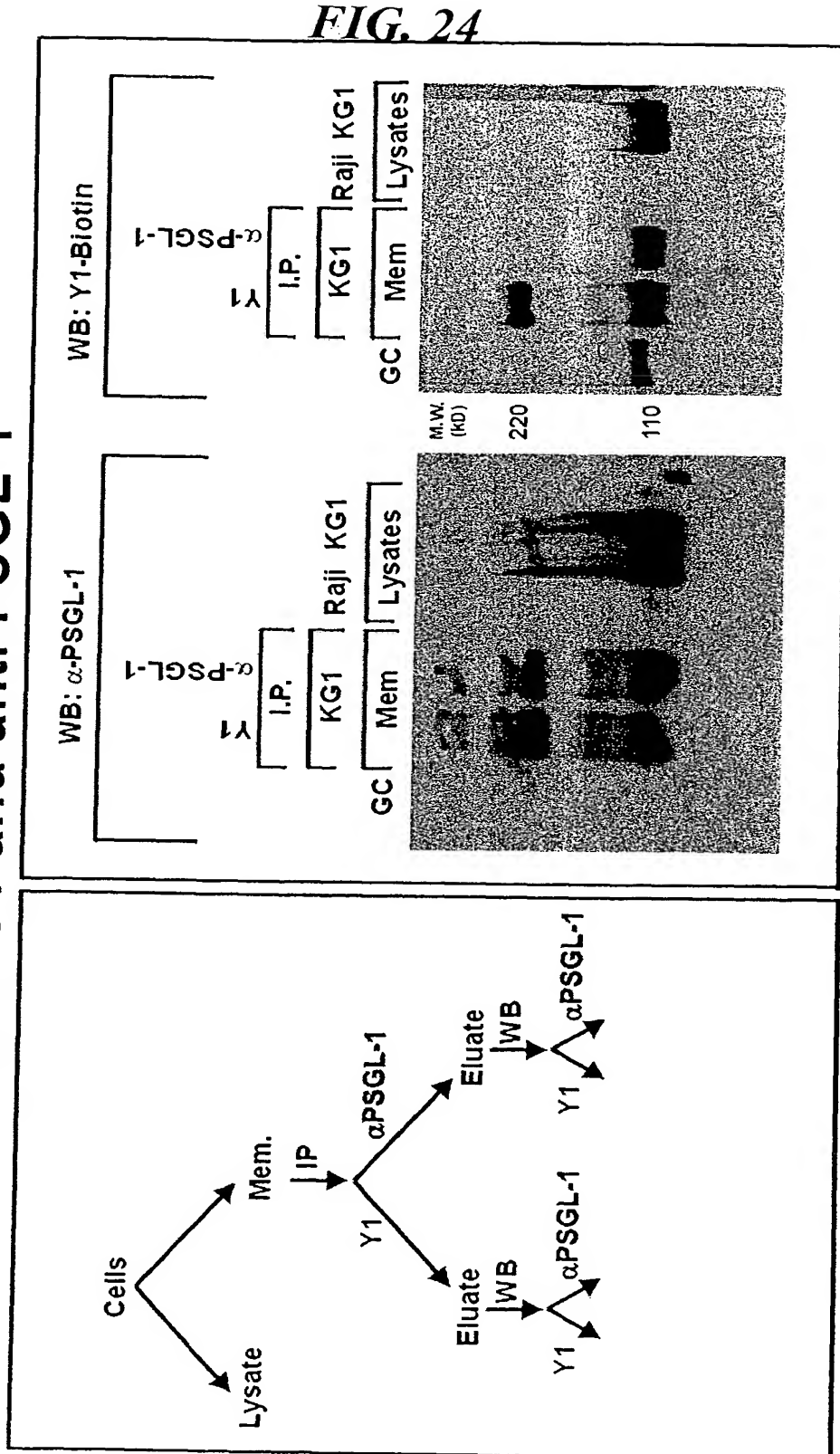
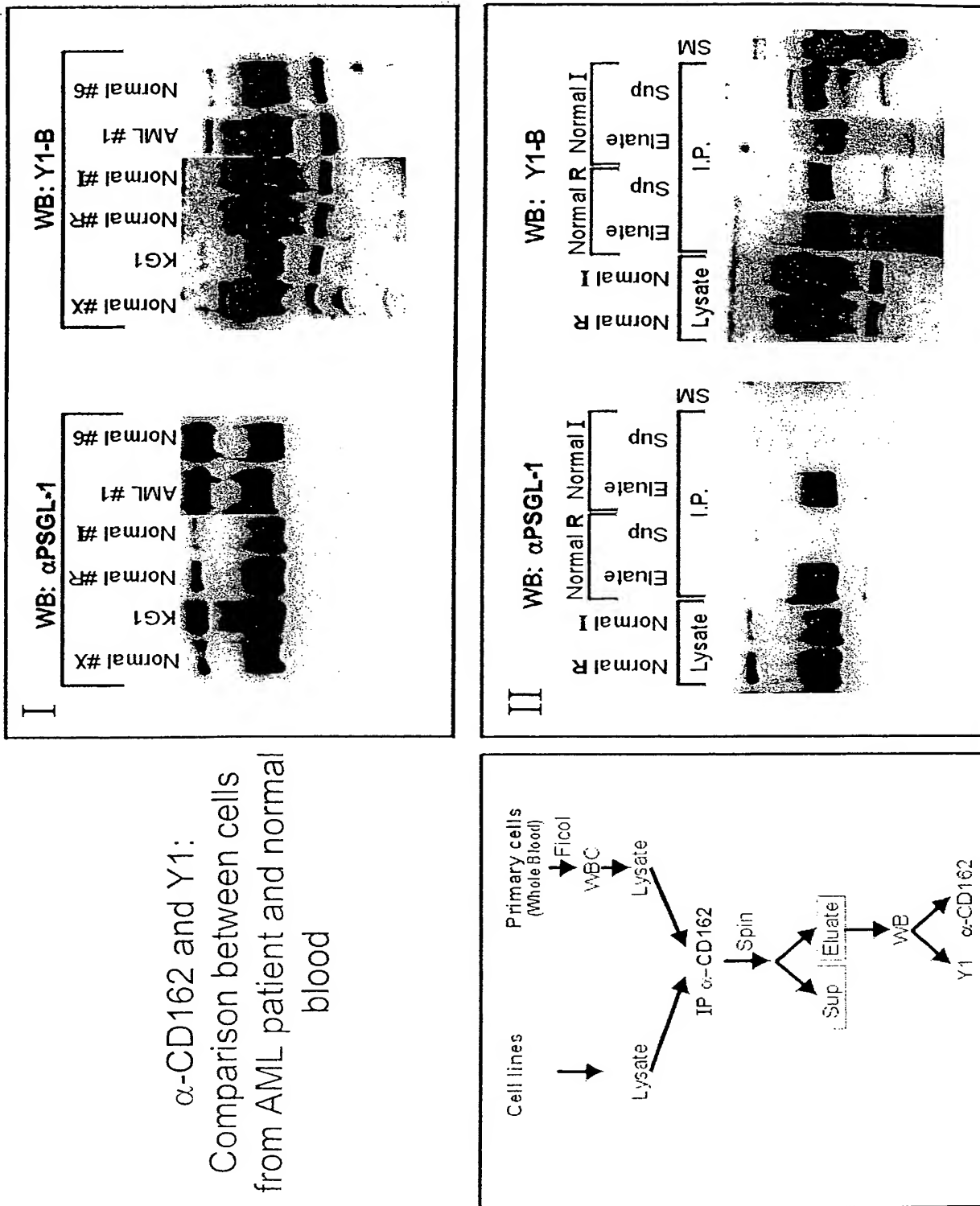


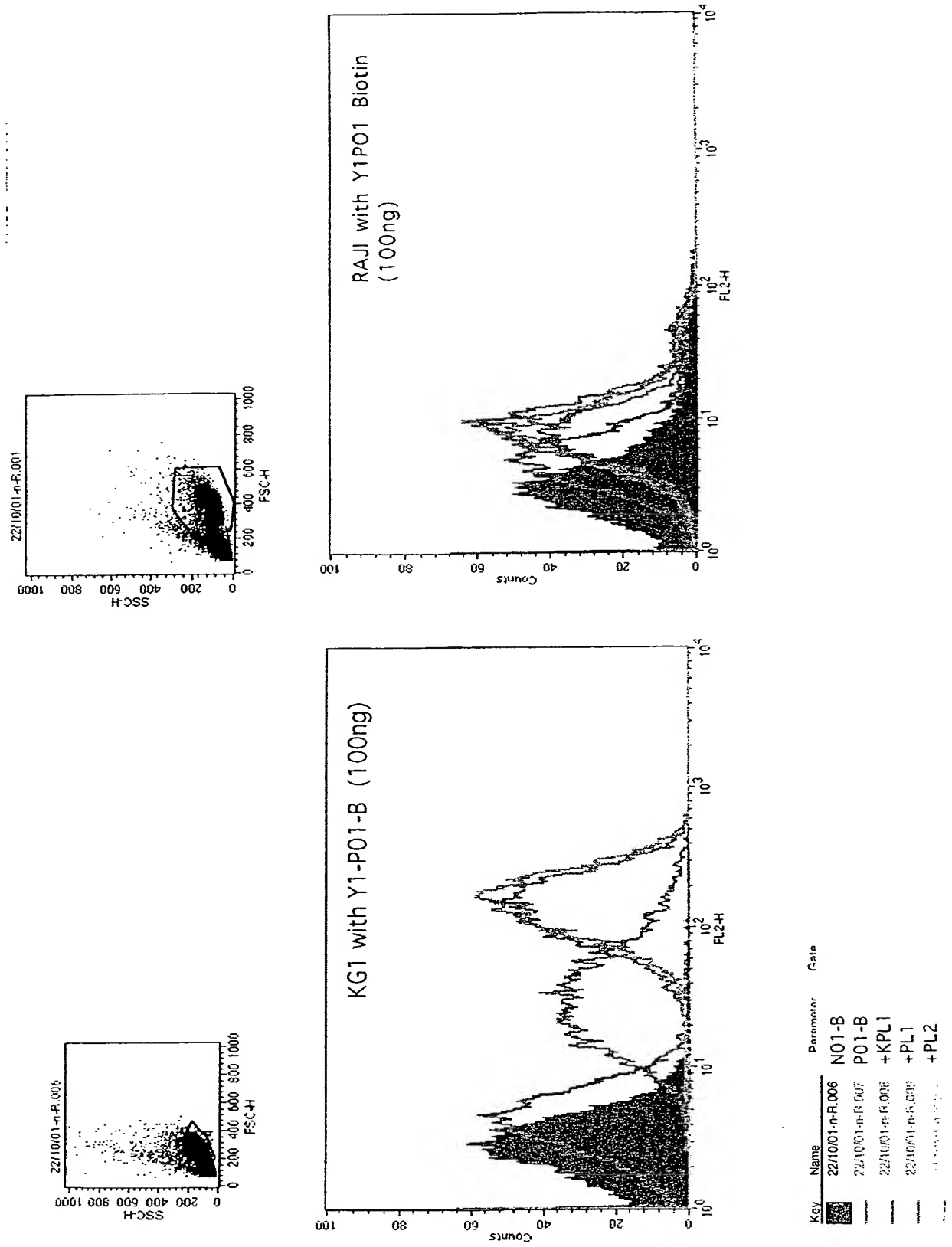


FIG. 25



$\alpha$ -CD162 and Y1:  
 Comparison between cells  
 from AML patient and normal  
 blood

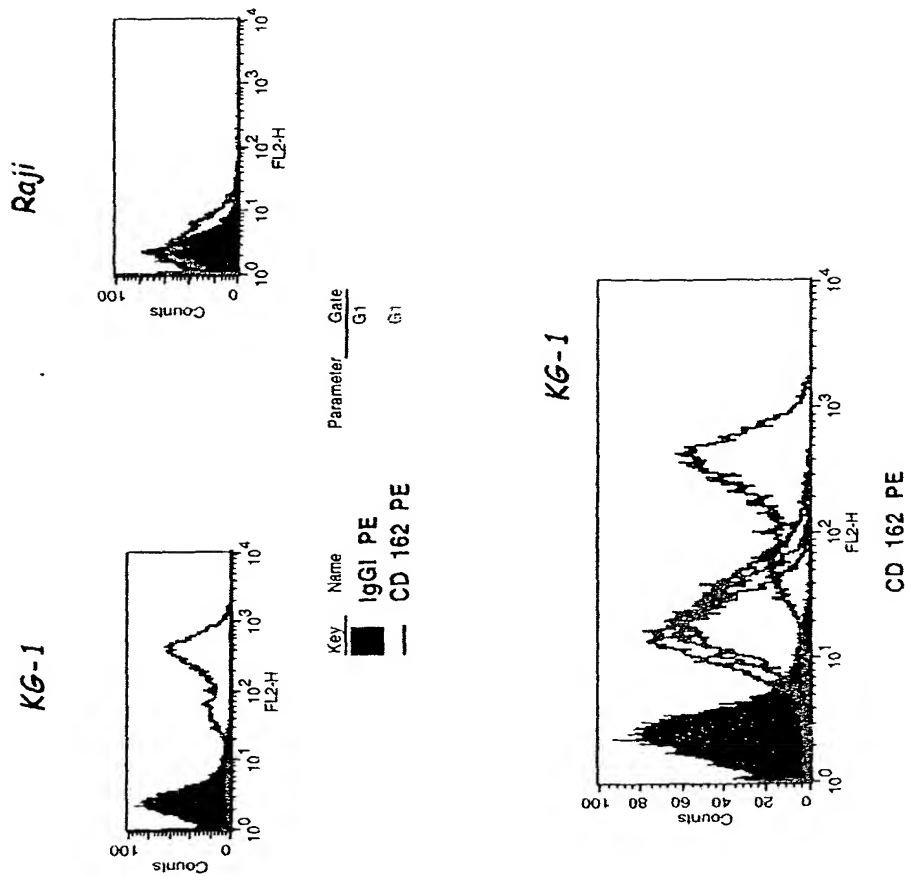
FIG. 26





# Specificity of Y1 Binding: Analysis by FACS

Binding of  
 $\alpha$  PSGL1  
( $\alpha$ CD162/KPL1);  
competition  
with Y1-IgG



KG-1

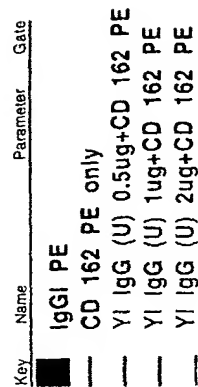
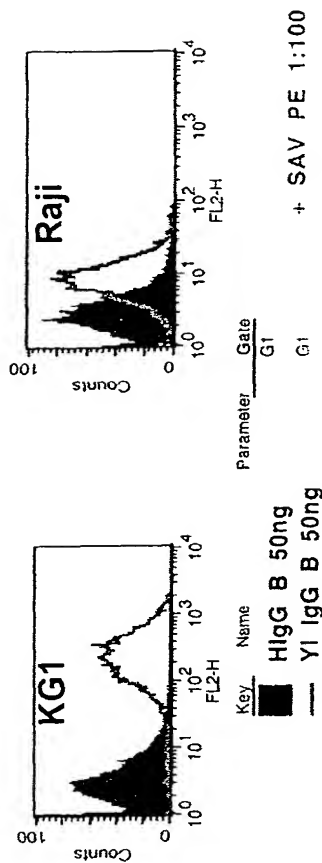


FIG. 27

# Specificity of Y1 Binding: Analysis by FACS

- Binding of  
 Y1-IgG;  
 competition  
 with  $\alpha$ PSGL-1  
 (CD162 /KPL1)



## COMPETITION

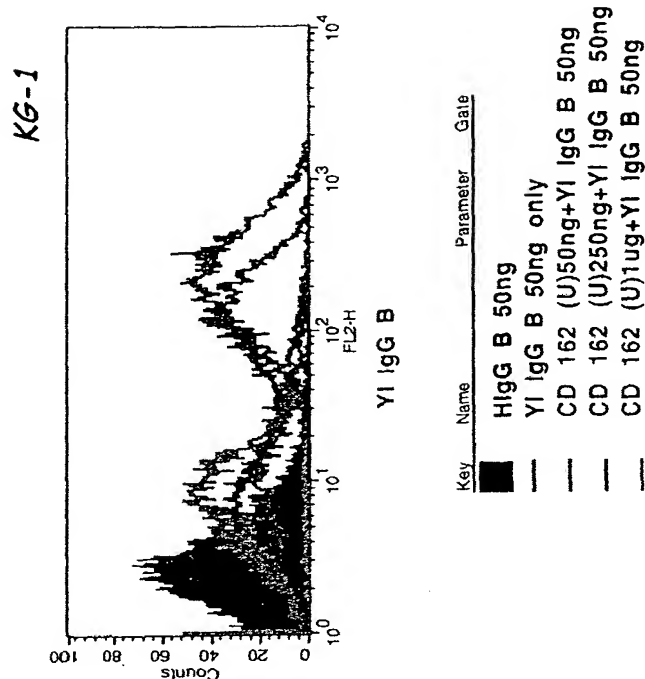
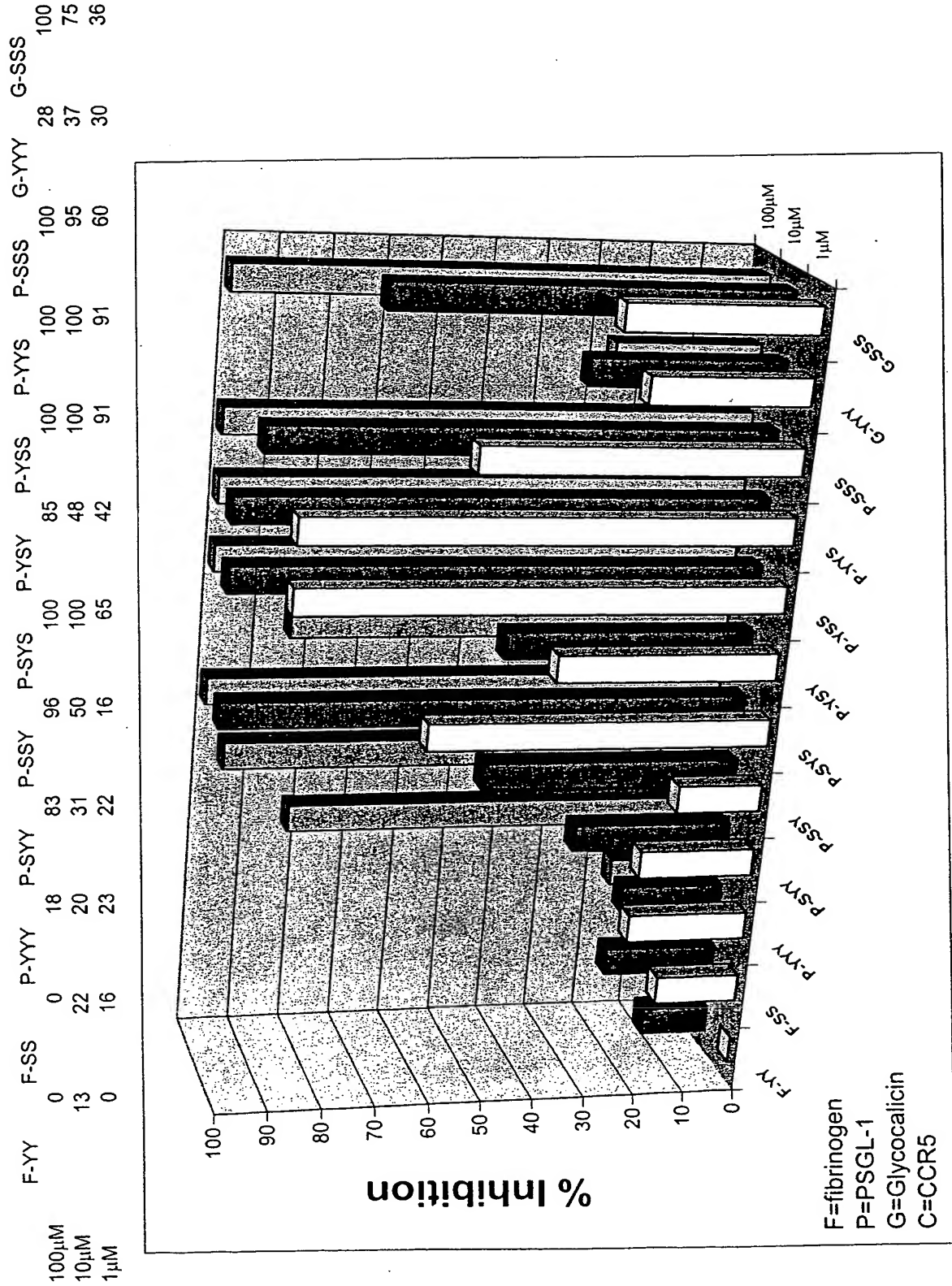


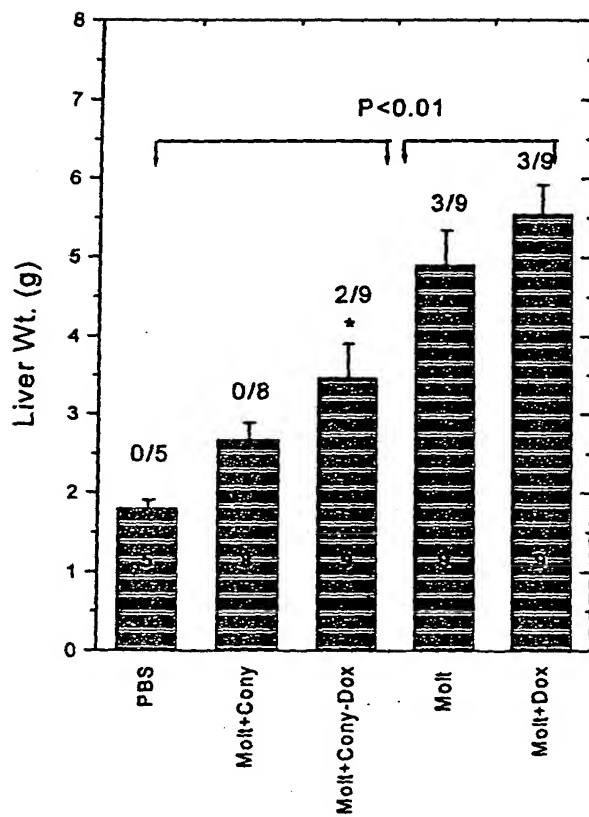
FIG. 28

FIG. 29





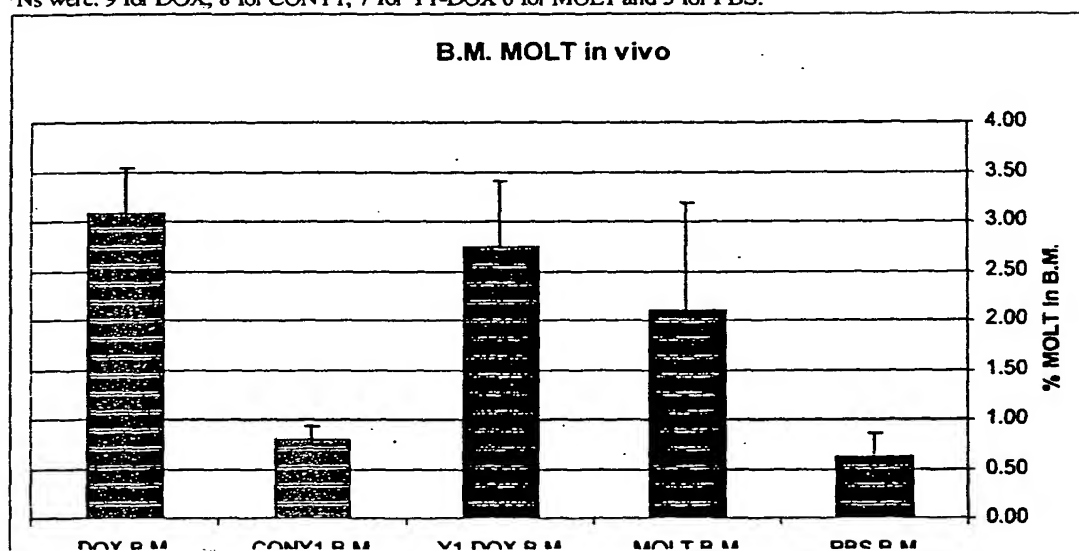
**FIG. 30**





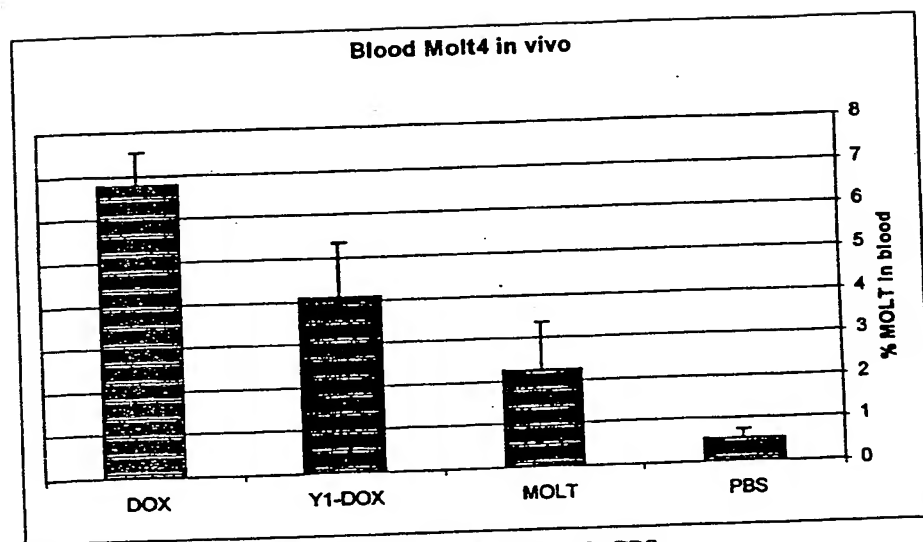
**FIG. 31**

\*Ns were: 9 for DOX, 8 for CONY1, 7 for Y1-DOX 6 for MOLT and 5 for PBS.





**FIG. 32**

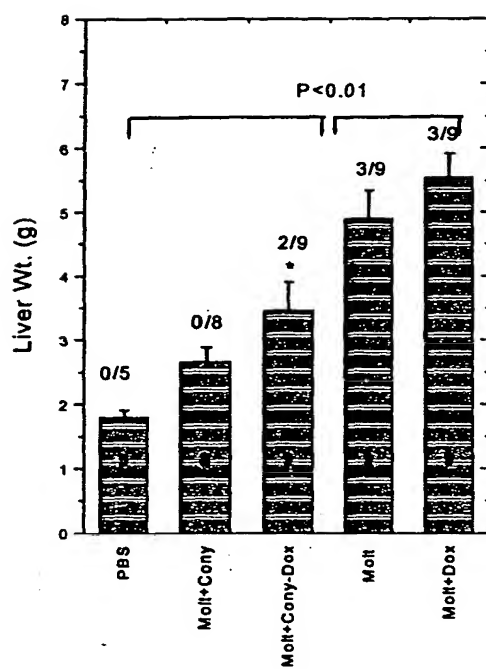


\*\*Ns were: 4 for DOX, 2 for Y1-DOX, 3 for MOLT and 3 for PBS.



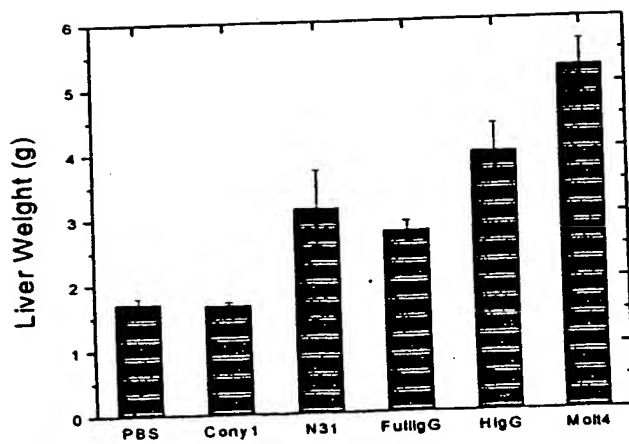


**FIG. 33**



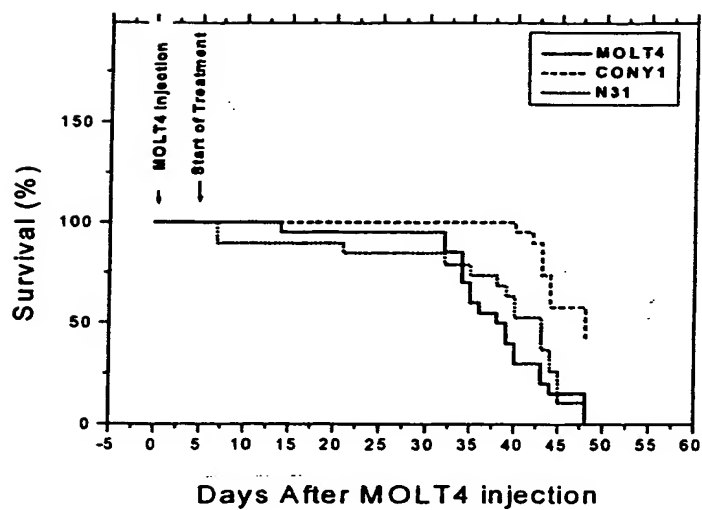


**FIG. 34**





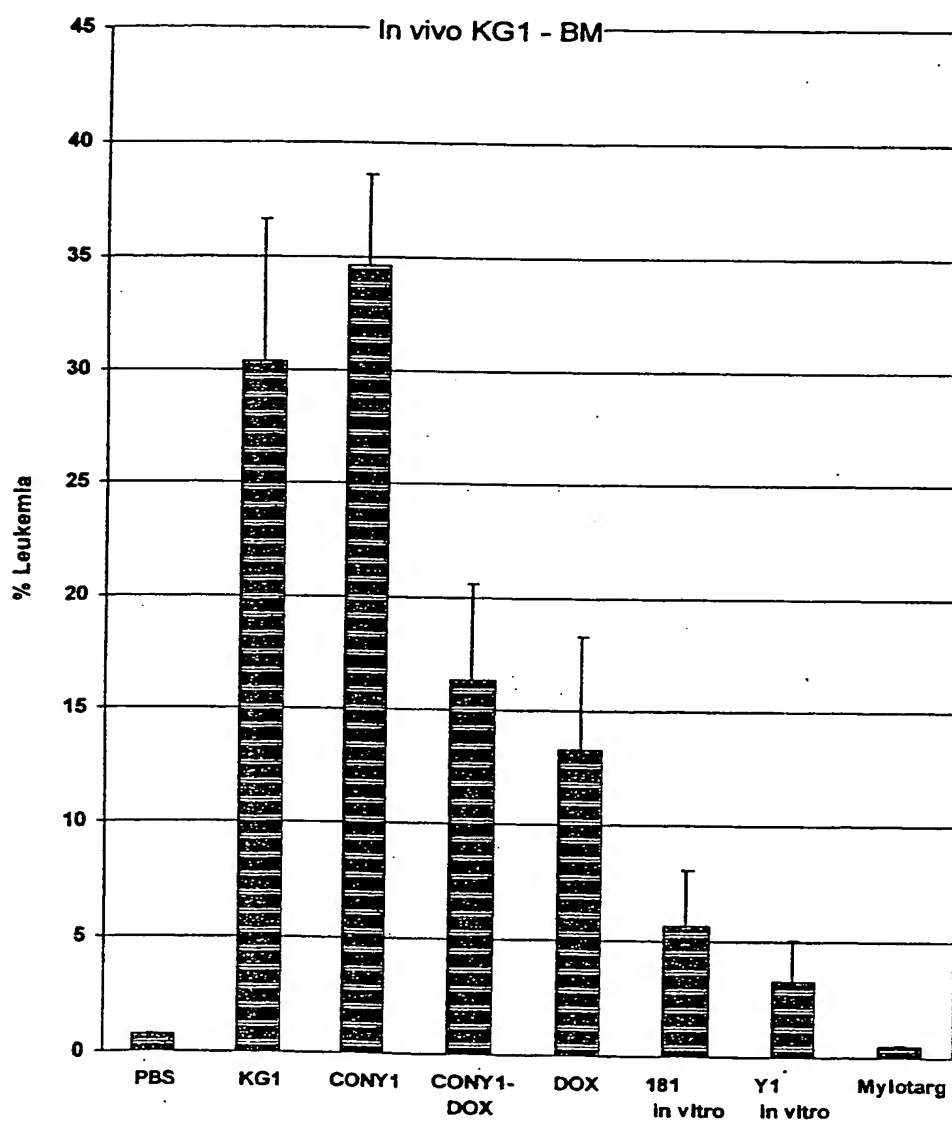
**FIG. 35**





**FIG. 36**

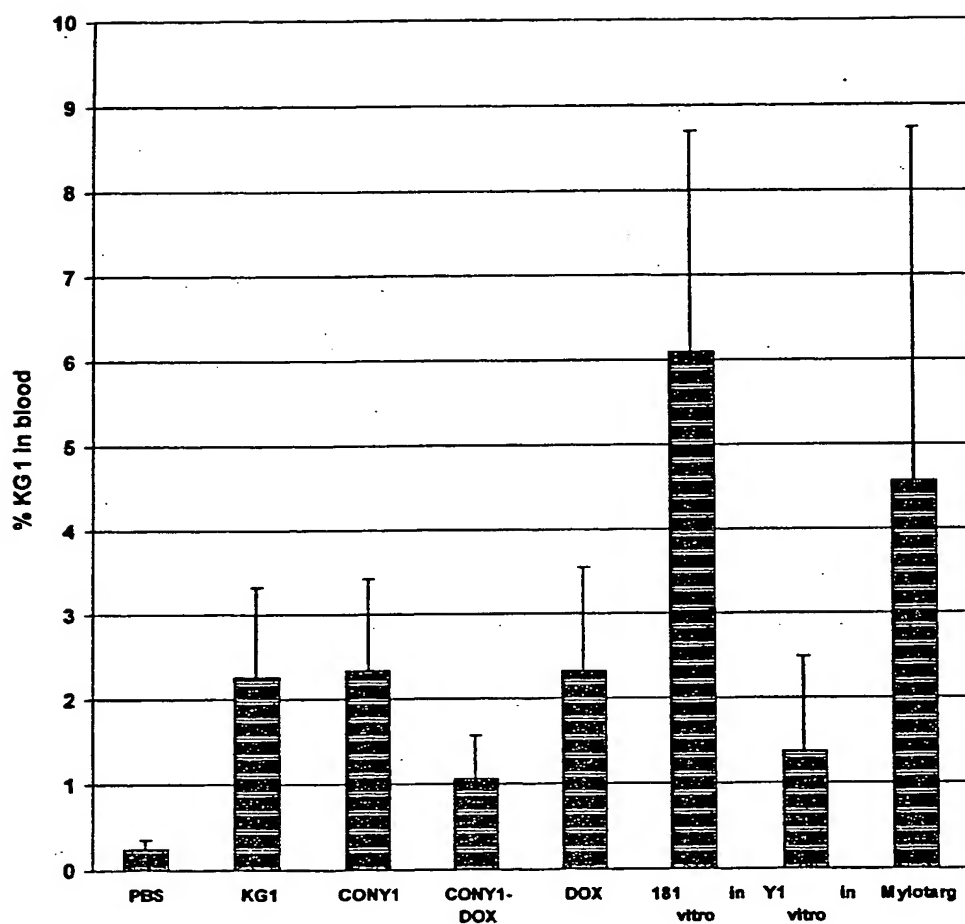
\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 11 for CONY1-DOX, 9 for DOX, 8 for 181 in vitro, 9 for Y1 in vitro and 9 for Mylotarg.





**FIG. 37**

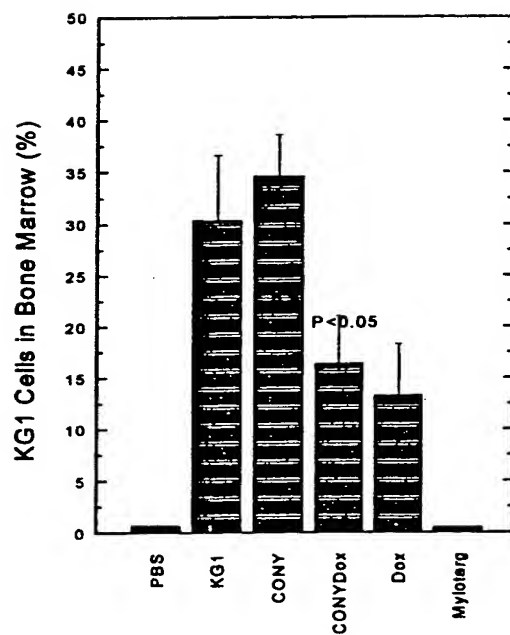
**In vivo KG1 - Blood**



\*\*\*\*Ns were: 8 for PBS, 9 for KG1, 8 for CONY1, 9 for CONY1-DOX, 11 for DOX (including one mice injected with 5mg/kg DOX), 7 for 181 in vitro, 8 for Y1 in vitro and 7 for Mylotarg.

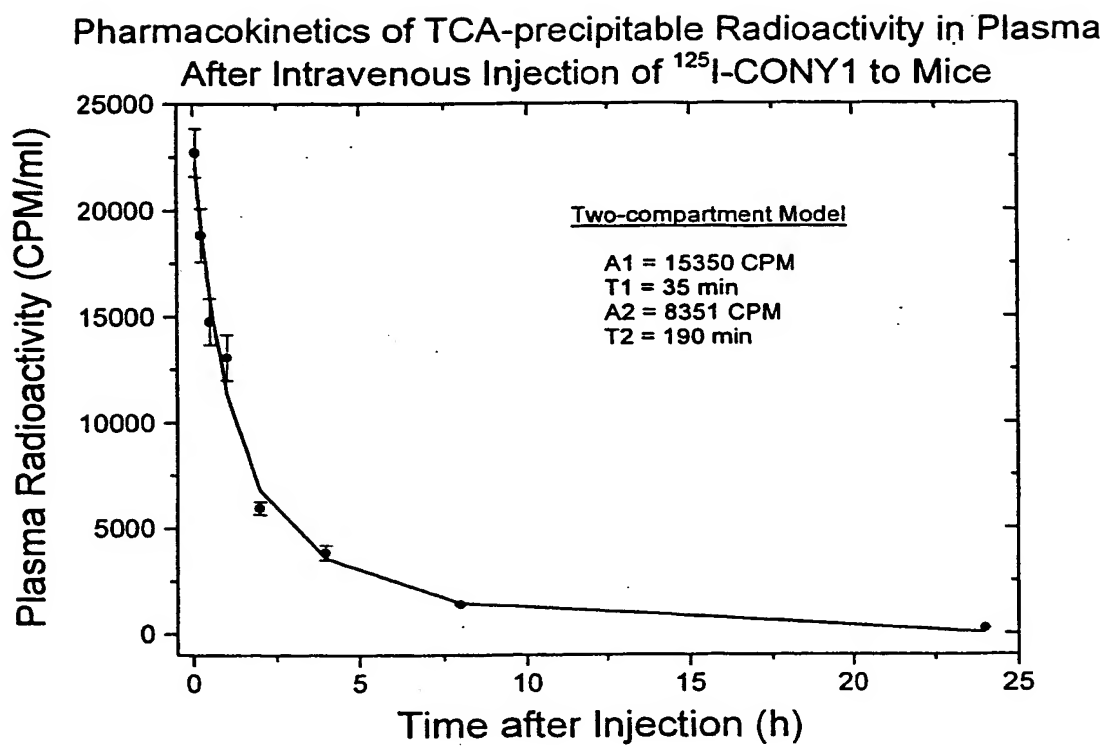


**FIG. 38**



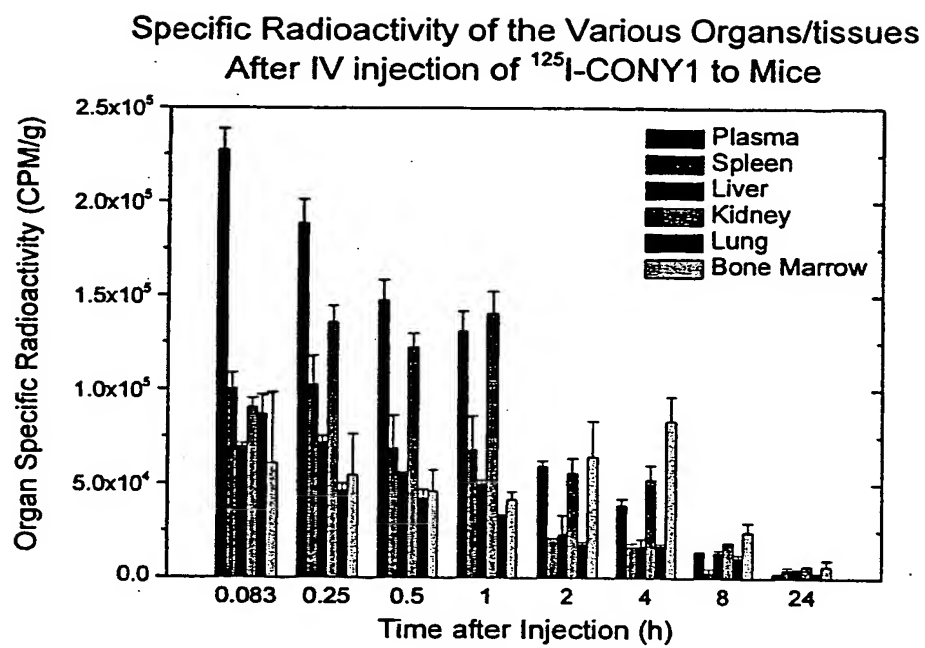


**FIG. 39**





**FIG. 40**

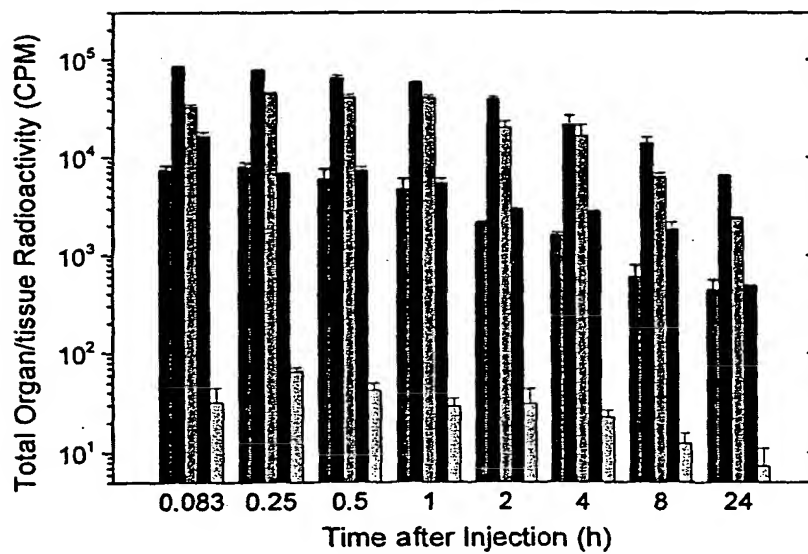






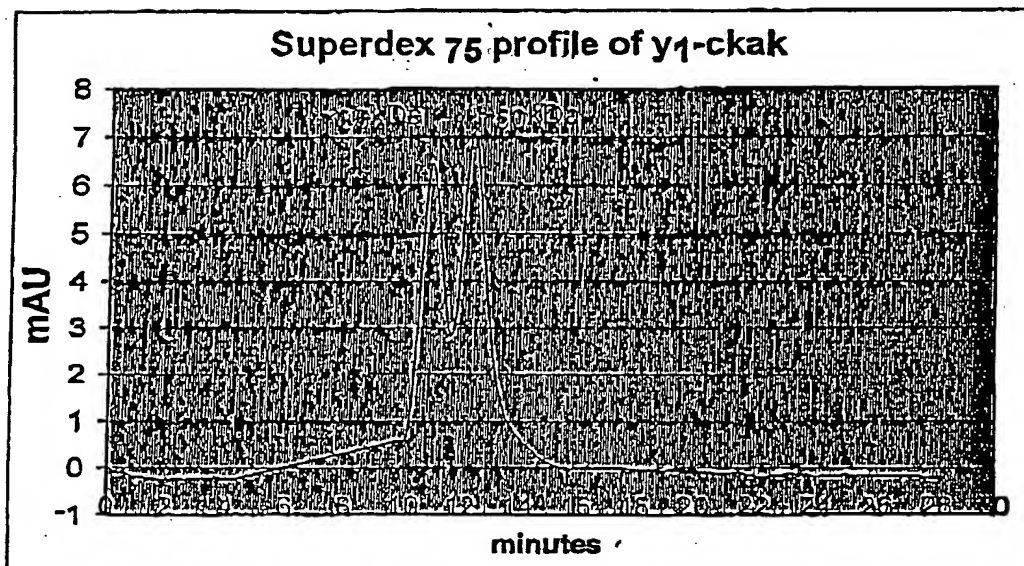
**FIG. 41**

Distribution of Radioactivity in Body organs after  
Injection of  $^{125}\text{I}$ -CONY1 to Mice



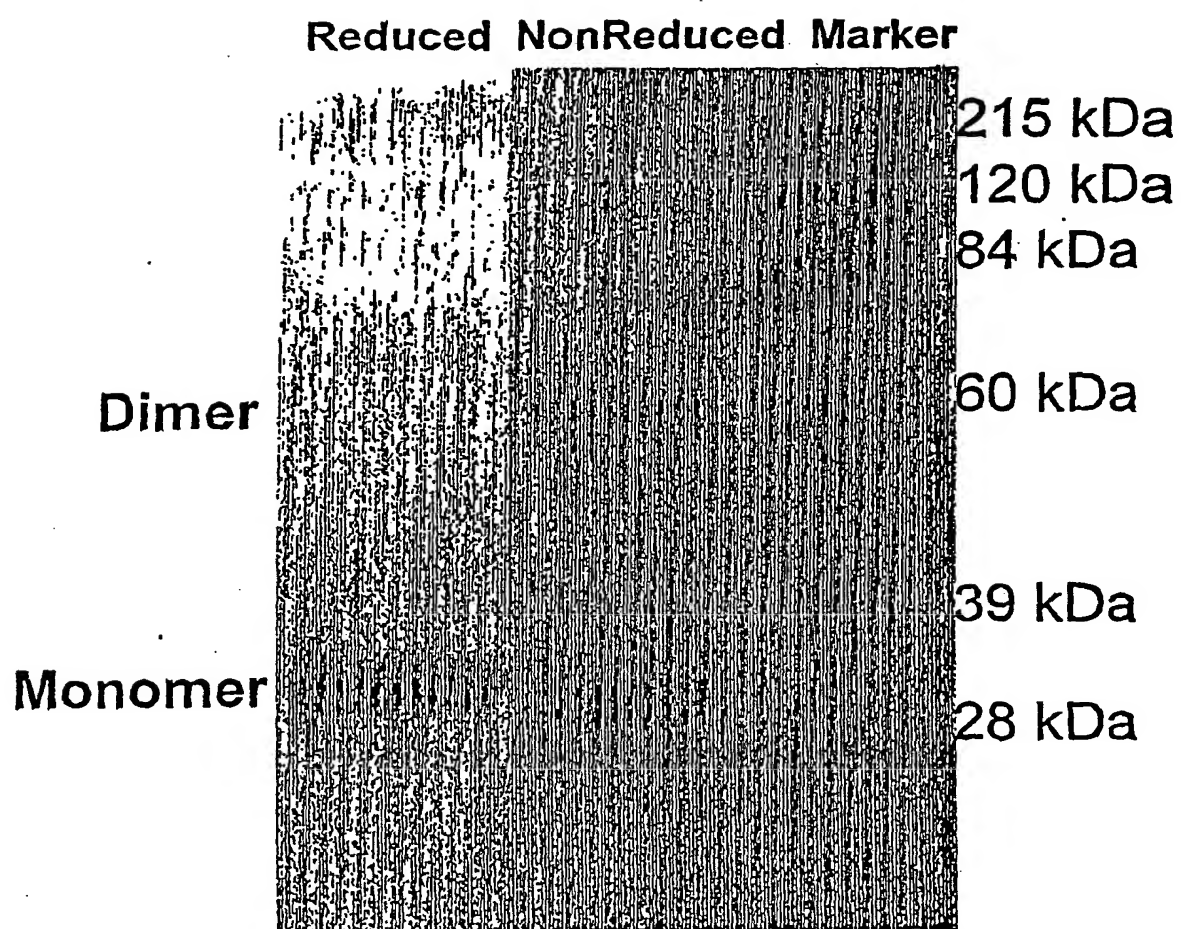


**FIG. 42**



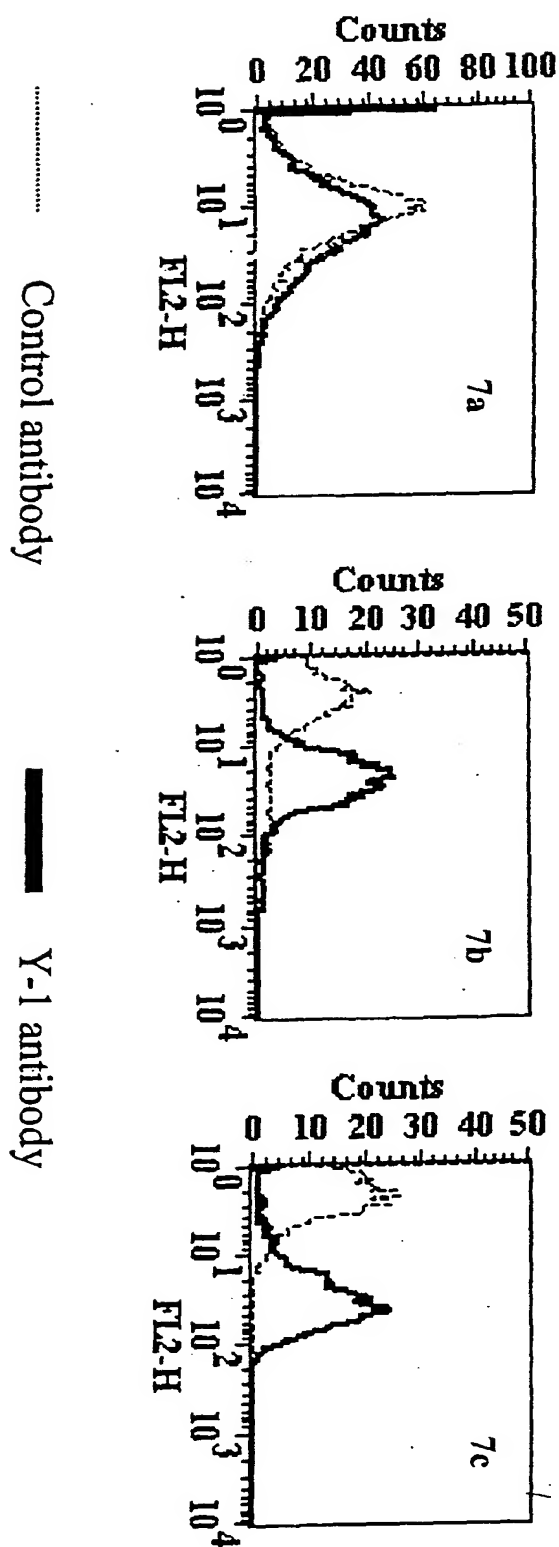


**FIG. 43**





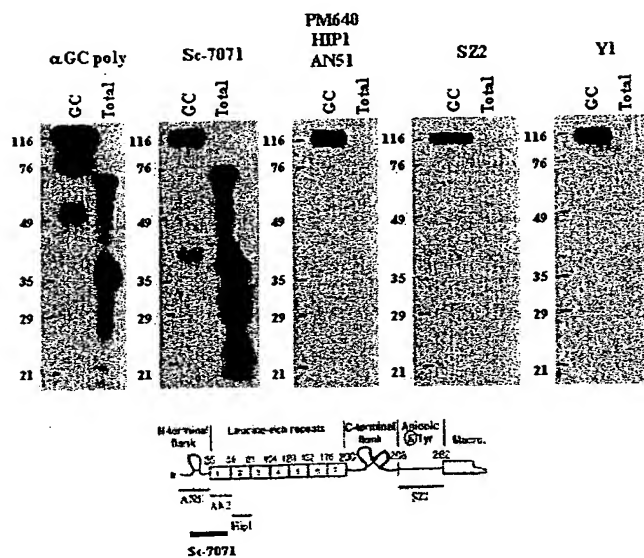
**FIG. 44**





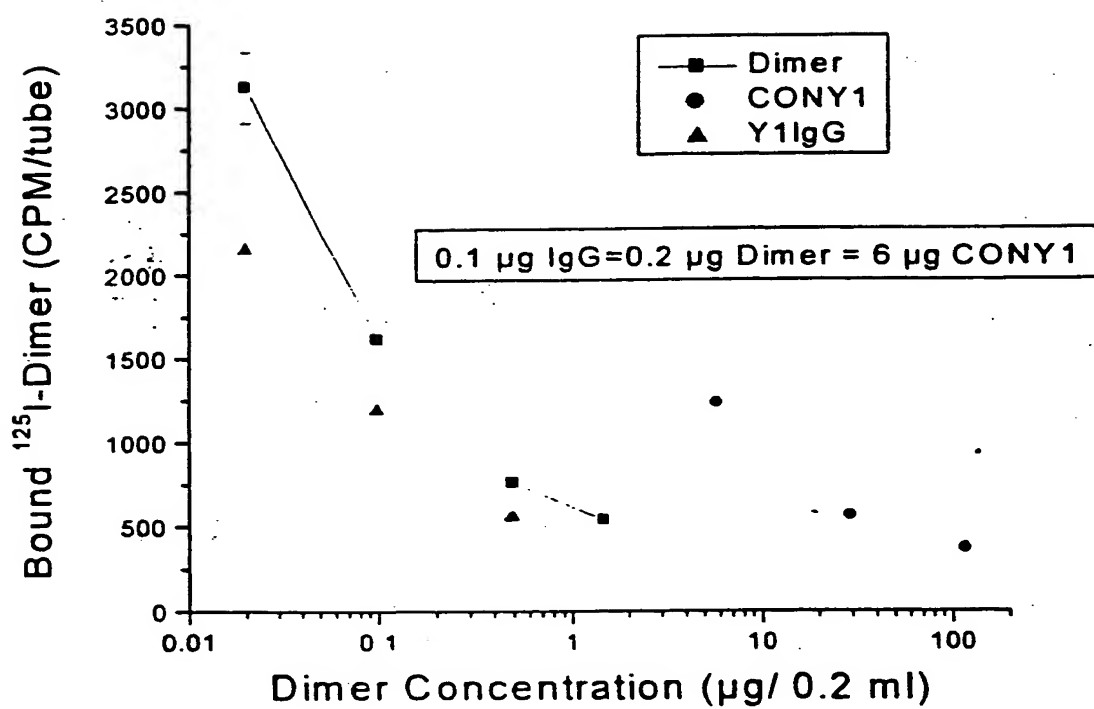
**FIG. 45**

**Epitopes of anti-GPIb $\alpha$  antibodies**





**FIG. 46**





**FIG. 47**

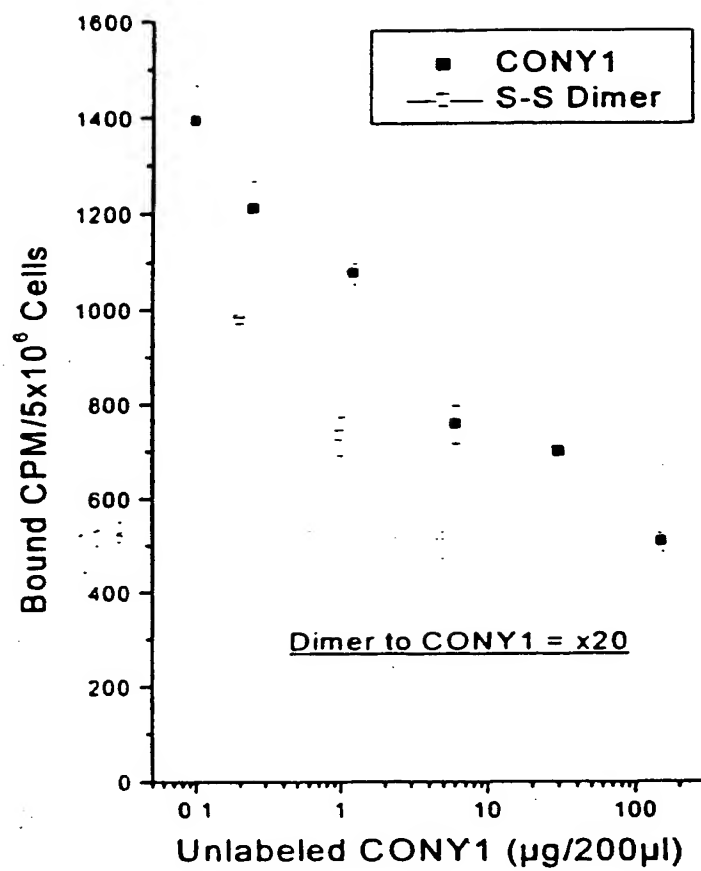




FIG. 48A: The ORF and Amino Acid Sequence of Y1-HC

SEQ ID NO: 205 (nucleic acid sequence): SEQ ID NO: 206 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1	M A W A L L L L T L L T Q D T G S W A D
61	ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21	I Q L V E S G G G V V R P G G S L R L S
121	TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGTCCA
41	C A A S G F T F D D Y G M S W V R Q A P
181	GGGAAGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGTTATGCA
61	G K G L E W V S G I N W N G G S T G Y A
241	GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAAGTCCCTGTATCTG
81	D S V K G R F T I S R D N A K N S L Y L
301	CAAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGATGAGGGCT
101	Q M N S L R A E D T A V Y Y C A R M R A
361	CCTGTGATTTGGGGCCAAGGTACCTTGGTCAACGTCTCGAGTGTCTCCACCAAGGGCCCA
121	P V I W G Q G T L V T V S S A S T K G P
421	TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141	S V F P L A P S S K S T S G G T A A L G
481	TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGCGCCCTG
161	C L V K D Y F P E P V T V S W N S G A L
541	ACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGC
181	T S G V H T F P A V L Q S S G L Y S L S
601	AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201	S V V T V P S S S L G T Q T Y I C N V N
661	CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAT
221	H K P S N T K V D K R V E P K S C D K T
721	CACACATGCCCACCGTGCCAGACCTGAACTCCTGGGGGACTGTGAGTCTTCOTCTTC
241	H T C P P C P A P E L L G G P S V F L F
781	CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTG
261	P P K P K D T L M I S R T P E V T C V V
841	GTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281	V D V S H E D P E V K F N W Y V D G V E
901	GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301	V H N A K T K P R E E Q Y N S T Y R V V
961	AGCGTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTC
321	S V L T V L H Q D W L N G K E Y K C K V
1021	TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCC
341	S N K A L P A P I E K T I S K A K G Q P
1081	OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361	R E P Q V Y T L P P S R E E M T K N Q V
1141	AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381	S L T C L V K G F Y P S D I A V E W E S
1201	AATGGGCAGCCGGAGAACAATAACAAGACCACGTCTCCCGTGTCTGGACTCCGACGGCTCC
401	N G Q P E N N Y K T T S P V L D S D G S
1261	TTCTTCCTCTATAGCAAGCTCACCGTGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421	F F L Y S K L T V D K S R W Q Q G N V F
1321	TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441	S C S V M H E A L H N H Y T Q K S L S L
1381	TCTCTGGGTAAATGA
461	S L G K *





FIG. 48B: The ORF and Amino Acid Sequence of Y1-LC

SEQ ID NO: 207 (nucleic acid sequence); SEQ ID NO: 208 (amino acid sequence)

1	ATGGCCTGGGCTCTGCTGCTCCTCACCCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1	<u>M A W A L L L L T L L T Q D T G S W A D</u>
61	GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21	A E L T Q D P A V S V A L G Q T V R I T
1212	TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41	C Q G D S L R S Y Y A S W Y Q Q K P G Q
181	CCCCCTGTACTTGTTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTTC
161	A P V L V I Y G K N N R P S G I P D R F
241	TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81	S G S S S G N T A S L T I T G A Q A E D
301	GAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATGTGGTATTTCGGCGGA
101	E A D Y Y C N S R D S S G N H V V F G G
361	GGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121	G T K L T V L G Q P K A A P S V T L F P
421	CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141	P S S E E L Q A N K A T L V C L I S D F
481	TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161	Y P G A V T V A W K A D S S P V K A G V
541	GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181	E T T T P S K Q S N N K Y A A S S Y L S
601	CTGACGCCTGAGCAGTGGAAGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGG
201	L T P E Q W K S H K S Y S C Q V T H E G
661	AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221	S T V E K T V A P T E C S *



## FIG. 49

1	11	21	31	41	51	
1	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	SYAMSWVRQA	PGKGLEWVSA	ISGSGGSTYY 60
61	ADSVKGRFTI	SRDNSKNTLY	LOMNSLRAED	TAVYYCARVA	KTLMROYSLV	GQGLVTVSR 120
121	GGGSGGGGS	GGGGSSELTQ	DPAVSVALGO	TVRITCQGDS	LSYYASWYQ	QKPGQAPVLV 180
181	IYGKNNRPSG	IPDRFSGSSS	GNTASLTITG	AQAEDEADYY	CNSRDSSGNH	VVFGGGTKLT 240
241	VLGAAAEQKL	ISEEDLNGAA				

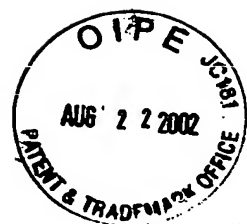


FIG. 50

	10	20	30	40	50	60
1	AtTaTTACTC	gCGGCCcAGC	CgGCCcAGC	CGAGGTGCAG	CTGGTGGAGT	CTGGGGGAGG
3	L L L A A Q P A M A	E V Q L V E S G G G				
	70	80	90	100	110	120
1	CTTGGTACAG	CCTGGGGGGT	CCCTGAGACT	CTCCTGTGCA	GCCTCTGGAT	TCACCTTTAG
3	L V Q P G G S L R L S C A A S G F T F S					
	130	140	150	160	170	180
1	CAGCTATGCC	ATGAGCTGGG	TCCGCCAGGC	TCCAGGGAAG	GGGCTGGAGT	GGGTCTCAGC
3	S Y A M S W V R Q A P G K G L E W V S A					
	190	200	210	220	230	240
1	TATTAGTGGT	AGTGGTGGTA	GCACATACTA	CGCAGACTCC	GTGAAGGGCC	GGTTCACCAT
3	I S G S G G S T Y Y A D S V K G R F T I					
	250	260	270	280	290	300
1	CTCCAGAGAC	AATTCCAAGA	ACACGCTGTA	TCTGCAAATG	AACAGCCTGA	GAGCCGAGGA
3	S R D N S K N T L Y L Q M N S L R A E D					
	310	320	330	340	350	360
1	CACGGCCGTG	TATTACTGTG	CAAGATCGG	TCAGATGTT	TAAGCTTAAT	GGGGCCAAGG
3	T A V Y Y C A R T G Q S I K R S W G Q G					
	370	380	390	400	410	420
1	TACCCTGGTC	ACCGTGTGCA	GAGGTGGAGG	CGGTTCAGGC	GGAGGTGGCT	CTGGCGGTGG
3	T L V T V S R G G G G S G G G G S G G G					
	430	440	450	460	470	480
1	CGGATCGTCT	GAGCTGACTC	AGGACCCTGC	TGTGTCTGTG	GcCTTGGGAC	AgACAGTCAG
3	G S S E L T Q D P A V S V A L G Q T V R					
	490	500	510	520	530	540
1	GATCACATGC	CAAGGAGACA	GCCTCAGAAG	CTATTATGCA	AGCTGGTACC	AGCAGAAGCC
3	I T C Q G D S L R S Y Y A S W Y Q Q K P					
	550	560	570	580	590	600
1	AGGACAGGCC	CCTGTACTTG	TCATCTATGG	TAAAAACAAC	CGGCCCTCAG	GGATCCCAGA
3	G Q A P V L V I Y G K N N R P S G I P I					
	610	620	630	640	650	660
1	CCGATTCTCT	GGCTCCAGCT	CAGGAAACAC	AGCTTCCTTG	ACCATCACTG	GGGCTCAGGC
3	R F S G S S S G N T A S L T I T G A Q F					
	670	680	690	700	710	720
1	GGAAGATGAG	GCTGACTATT	ACTGTAACTC	CCGGGACAGC	AGTGGTAACC	ATGTGGTATT
3	E D E A D Y Y C N S R D S S G N H V V I					
	730	740	750	760	770	780
1	CGGCGGAGGG	ACCAAGCTGA	CGTCCTTAGG	TGCGGCCGCA	GAACAAAAAC	TCATCTCAGT
3	G G G T K L T V L G A A A E Q K L I S I					
	790	800	810	820	830	840
1	AGAGGATCTG	AatGGGGCOG	CAGGAACTG	TtGAATTTT	TAAGTTAac	T
3	E D L N G A A * N C * I F * V N					

1/16 SEQ ID NO: 210



## ***FIG. 51***

Sequence of Y1-Biotag (SEQ ID NO: 211)

1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDY'GMSWVRQ  
41 APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL  
81 YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LVTVSRGGGG  
121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY  
161 YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA  
201 SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG  
241 GGLNDIFEAQ KIEWHE



## ***FIG. 52***

Y1-cys-kak scFv (SEQ ID NO. 212)

I MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ  
APGKGLEWVS GINWNGGSTG 60

61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM  
RAPVIWGQGT LVTVSRGGGG 120

121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY  
YASWYQQKPG QAPVLVIYGK 180

181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR  
DSSGNHVVFG GGTKLTVLGG 240

241 GGCKAK